

Dispensing for  
Pharmaceutical Students



COPPER AND GUNN'S

# Pharmacy for Pharmaceutical Students

*Revised by*

Colin Gunn B.PHARM., F.P.S.

*Head of the School of Pharmacy Leicester College of Technology  
Formerly Lecturer in Pharmaceutics in the above College and  
Demonstrator in Pharmaceutics etc Heriot-Watt College Edinburgh  
Examiner in Pharmaceutics to the Pharmaceutical Society  
of Great Britain and sometime Examiner in Pharmaceutics to the  
Queen's University Belfast*

*With a section on Sterilisation Practice by*

S. J. Carter B.PHARM., F.P.S.

*Principal Lecturer in Pharmaceutics School of Pharmacy Leicester  
College of Technology Examiner in Pharmaceutics to the Pharma-  
ceutical Society of Great Britain*

Eleventh Edition



London

Pitman Medical Publishing Co. Ltd

<i>First edition 1929</i>	<i>Reprinted 1939</i>
<i>Second edition 1930</i>	<i>Seventh edition 1941</i>
<i>Reprinted 1931</i>	<i>Reprinted 1944</i>
<i>Third edition 1933</i>	<i>Eighth edition 1947</i>
<i>Fourth edition 1934</i>	<i>Ninth edition 1948</i>
<i>Fifth edition 1936</i>	<i>Tenth edition 1949</i>
<i>Sixth edition 1937</i>	<i>Reprinted 1956</i>
<i>Eleventh edition 1965</i>	

PITMAN MEDICAL PUBLISHING COMPANY LTD  
46 Charlotte Street, London, W1

*Associated Companies*

SIR ISAAC PITMAN AND SONS LTD  
Pitman House, Parker Street, Kingsway,  
London, W.C.2

The Pitman Press, Bath  
Pitman House, Bourke Street, Carlton, Melbourne  
22-25 Beckett's Buildings, President Street,  
Johannesburg

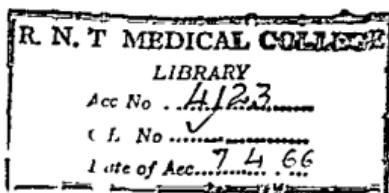
PITMAN PUBLISHING CORPORATION  
2 West 45th Street, New York

SIR ISAAC PITMAN AND SONS (CANADA) LTD  
(Incorporating the Commercial Text Book Company)  
Pitman House, 381-383 Church Street, Toronto

5.4

600-65

© Pitman Medical Publishing Co Ltd 1965





# Contents

PREFACE page vii

**Part One**  
**General Dispensing**

1 <i>Introduction</i>	3	11 <i>Suppositories, Pessaries Urethral and Nasal Bougies</i>	166
2 <i>Weights and Measures</i>	9	12 <i>Inhalations Spray Solutions and Throat Paints</i>	181
3 <i>Calculations</i>	15	13 <i>Eye Drops and Ear Drops</i>	184
4 <i>Mixtures</i>	40	14 <i>Gelatin Capsules</i>	196
5 <i>Emulsions</i>	55	15 <i>Pastilles and Lozenges</i>	198
6 <i>Powders</i>	78	16 <i>Incompatibility</i>	202
7 <i>Pills</i>	93	17 <i>The Dispensing of Proprietaries</i>	215
8 <i>Compressed Tablets</i>	97	18 <i>Medical Gases</i>	217
9 <i>Lotions Liniments and Applications</i>	122		
10 <i>Ointments Creams Pastes and Jellies</i>	133		

**Part Two**  
**Sterilisation Practice**

19 <i>Microbiological Aspects of Sterilisation Processes</i>	222	24 <i>Aseptic Technique</i>	400
20 <i>The Formulation of Injections</i>	237	25 <i>Sterility Testing</i>	446
21 <i>Containers and Closures</i>	274	26 <i>Aseptic Processing</i>	479
22 <i>Sterilisation by Heat</i>	309	27 <i>Gaseous Sterilisation</i>	517
23 <i>The Preparation of Heat sterilised Injections</i>	354	28 <i>Sterilisation by Radiations</i>	526
		29 <i>Sterilisation of Equipment and other Articles</i>	535

**Appendixes**

1 <i>Reducing Agents and Antioxidants</i>	549	5 <i>Classification of Injections under Methods of Stabilisation</i>	560
2 <i>Pyrogen Tests</i>	554	6 <i>Latin Terms used in Prescriptions</i>	564
3 <i>Incompatibilities of Bactericides</i>	557	7 <i>Posology</i>	573
4 <i>Classification of Injections under Methods of Sterilisation</i>	558	8 <i>Answers to Calculations</i>	592

INDEX page 595

## Acknowledgements

The use of portions of the text of the *United States Pharmacopoeia*, XVIth Revision, official October 1, 1960, is by permission received from the Board of Trustees of the United States Pharmacopoeial Convention. The said Board is not responsible for any inaccuracies of the text thus used

Material from British Standards is reproduced by permission of the British Standards Institution, 2 Park Street, London, W 1 from whom official copies of the complete Standards may be purchased.

The authors of pharmaceutical textbooks must lean heavily on the experience of practising pharmacists. We gratefully acknowledge the help we have received from almost every pharmaceutical house in the country and from many hospital and retail pharmacists. Thanks are also due to the pharmaceutics staff of the Leicester School of Pharmacy, in particular, Mr Harold Fowler, for fruitful discussions on technological aspects of sterilisation procedures and for supplying Fig 8.9, Mr Brian Griffiths for Fig 23 12, and Mr Arthur Boyall, for practical help in many directions. The author of Part 2 owes a great debt of gratitude to his wife who corrected the copy, organised and checked the references and supplied many of the unacknowledged illustrations.



## Preface

AN important feature of the eleventh edition of this book is the more extensive development of the theoretical principles underlying practical procedures. One result has been a redistribution of material between this book and its companion volume *Tutorial Pharmacy*. In future, disinfection and disinfectants will be included in the latter which will also contain the new subject of radioisotopes. The chapter on tablets has been retained here for the sake of continuity, although it will be transferred to the next edition of *Tutorial Pharmacy*, where it will occur with other manufacturing processes.

The highly practical slant of previous editions has been maintained but students should appreciate that it is an important part of their training to examine techniques critically and attempt to devise improvements. Often, e.g. in aseptic technique, there are equally good alternative procedures.

Although the Apothecaries System of weights and the Imperial System of measures are being gradually abandoned by pharmacists, many calculations in these systems have been retained since the need for such calculations will be with us for some time to come. To emphasise the importance of an ability to perform quick and accurate calculations, details which were formerly included as appendices have been brought forward to the beginning of the book.

The chapter on pills has been greatly reduced since pills are rarely prepared extemporaneously today, and no details of hand plaster making are included for the same reason. On the other hand, the chapters on eye drops and compressed tablets have been enlarged.

The section on sterilisation practice has been greatly extended to reflect more adequately its importance to the pharmacist, who can rightly claim to be the expert in this field.

In the preface to the tenth edition attention was drawn to the reduced emphasis on dispensing in pharmaceutical courses and that a relative incompetence in the subject, coupled with a somewhat superior attitude to it, is no rare thing among senior pharmacy students whose ability in the more scientific aspects of pharmacy is satisfactory. It cannot be too often repeated that the acquisition of manipulative skill and habits of precision and cleanliness are qualities which should be possessed by anyone with scientific aspirations.

School of Pharmacy,  
Leicester College of Technology

Colin Gunn  
S J Carter

PART ONE  
General Dispensing





# Introduction

## How to use this Book

THE student unacquainted with dispensing should begin by thoroughly mastering the tables of weights and measures given in Chapter 2. Some knowledge of Latin terms is also required from the beginning, and, unless familiar with prescription Latin, the student should read Appendix 6 at the outset, and then thoroughly learn two or three of the succeeding pages each week.

Within a week or two of beginning the course of dispensing, the student should start systematic work on 'Calculations', Chapter 3, and 'Posology', Appendix 7. With the former, one exercise each fortnight, with Exercise 3.9 divided over three such periods, will ensure that progress with certain of the dispensing exercises is not impeded by lack of knowledge of the calculations involved. Thorough mastery of a page of the Posological Tables each fortnight will cover this section in good time.

The questions set under 'Suggestions for Private Study' should be attempted without reference to the book. The examination papers of the Pharmaceutical Society frequently contain questions on dispensing, hence the questions set in this book will not only test the knowledge gained, but will also afford practice in preparing suitable answers.

The practical exercises set for revision should also be attempted without referring to the book.

## Observance of Laboratory Rules

Adherence to certain rules is insisted upon in most laboratories. The maxims and rules which follow are strictly observed in almost all dispensing laboratories.

- 1 **Accuracy** Faithful interpretation of the prescriber's intention is the dispenser's first duty. Carelessness, and any tendency to approximate, even in the apparently trivial acts of dispensing, is on no account permitted. (See p 21.)
- 2 **Cleanliness** It may appear superfluous to insist upon cleanliness in good dispensing, unfor-

tunately speed is sometimes cultivated at the expense of cleanliness, and it should be firmly checked. It is sometimes remarked that, as students' medicines will not be used, they can be prepared in desultory fashion. This fallacy can not be too strongly condemned, and every exercise must receive the same meticulous care as if it were a doctor's prescription for an actual patient.

- 3 **Balances** These must be checked before each act of weighing. The dispensing balance, weights, and drawer must be kept scrupulously clean, and each student should regard this as a personal responsibility.

Accurate chemical balances, preferably with counter balanced glass pans, must be used for weighing substances with a dose of 1 grain or less. The glass pans must not, under any consideration, be removed from the immediate vicinity when weighed. The pan should be promptly cleaned, returned to the balance, and the latter checked. It should become a habit to use forceps for all metric and Imperial grain weights.

- 4 **Checking Poisons** The demonstrator should be asked to check all poisons as soon as weighed or measured, and to initial the checking in the student's book.

- 5 **Replacing Stock Bottles** The tendency to accumulate bottles on the bench when they are no longer required for the prescription should be checked before it becomes a habit. It is slovenly, anti-social, and a hindrance to smooth working, particularly where several workers are employed.

## The Laboratory Notebook

A well kept practical book is of great value in revising and for future reference, particularly if it is well indexed. These books may be used for reference in the examinations of the Pharmaceutical Society, and this can be helpful. The student should be warned, however, that precious time can be wasted by excessive reference to books during examinations.

A suitable method of recording laboratory exercises in dispensing appears on pp 6 and 7, showing left- and right hand pages of the notebook. Particular attention should be given to the following—

- (a) The left hand page should be kept for actual prescriptions
- (b) The right hand page should have a margin.
- (c) Descriptions should record work done, i.e. they should be worded in the past tense
- (d) Spacing should be liberal, thus allowing for subsequent notes and references to be inserted
- (e) Wherever possible, a laboratory notebook should be indexed as the work progresses

#### General Dispensing Procedure

The student should memorise the following order of work, which, whenever appropriate, should be adopted throughout the course

- (a) Copy the prescription on the left hand page of the note-book, reserving the opposite page for a translation of the prescription, calculations, and details of procedure
- (b) Write the label for the preparation. The title, i.e. 'The Mixture,' 'The Draught,' etc., must be placed symmetrically at the top of the label, followed by the directions. Words should be used throughout, to avoid possible misreading of figures

The patient's name is written in the lower right hand portion of the label, and the number of the prescription on the left. In the following exercises these should be replaced by the student's name and the exercise number

- (c) Dispense the prescription
- (d) Complete the entry of the exercise in the note-book, as shown on pp 6 and 7, using the past tense because the description relates to a completed task.
- (e) Re-read the prescription memorising ingredients, quantities, and directions. With practice it is easy to give an accurate verbal description of the preparation, without referring to the book, and this habit should be cultivated from the start, as it will be of considerable value in many ways

- (f) Have the exercise checked by the demonstrator before proceeding with the next. Develop a critical attitude to each dispensed product. The student should set himself a high standard of personal achievement and never be satisfied with anything less. Opportunities will usually be found for repeating unsatisfactory products

#### Sources of Errors in Dispensing

The student must realise that his training in dispensing if approached with the proper attitude of mind, should eventually ensure that he becomes virtually incapable of making a serious error. This desirable end, however, can be attained only by the development of a highly responsible attitude to even the simplest task.

*Lack of Concentration.* This is one of the chief causes of error in dispensing. The work should not be carried out while engaged in a conversation or when the mind is struggling with other problems. One's whole attention must be given to the dispensing operation in hand, no matter how simple.

*Checking.* The name on the container of the substance being weighed or measured should be checked when the container is removed from and when it is returned to the shelf.

Quantities of poisons should be checked by a second responsible individual.

Failure deliberately to check calculations of quantities to be weighed or measured is a serious dispensing misdemeanour. Students should develop a routine for checking when calculating quantities and when transcribing formulae. When the ingredients in a formula add up to say, 1,000 grammes or millilitres, the quantities for any fraction of this amount should be added as a check, e.g. one fifth of the quantity would add up to 200. Formulae which are adjusted to volume with a vehicle cannot be checked in this way but the quantity of each ingredient may be compared with the others by noting the ratio of one to the other.

A labelling error may be as serious as a gross miscalculation. The label should always be written before the dispensing is done and carefully checked before affixing.

*Recording.* When recording in a practical book it is of considerable help to write and arrange the figures clearly and neatly. When writing a formula the quantities should be arranged in an orderly vertical column. In the metric system errors caused by misplacing the decimal point are avoided by so doing. Some dispensers prefer to use a vertical line in place of the decimal point and this is a method to be recommended.

*Arithmetical Slips.* It is a curious fact that students who have become reasonably skilled in mathematics are capable of making unaccountable slips in simple arithmetic. Only careful concentration, and the realisation that even the simplest operation requires one's whole attention, will avoid such errors.

*Weighing.* Weights should be checked when

removing them from their box and when returning them after the weighing has been completed Students should have an approximate idea of the densities of materials being weighed and, therefore, have some idea that the quantity on the balance pan is approximately correct Gross errors can be avoided in this way

### Sources of Inaccuracies in Dispensing

Under this heading are discussed the inaccuracies which are due to manipulation and the quality of the apparatus used

**Weighing** Various types of dispensing balances are used and it is necessary for the student to familiarise himself with the instrument or instruments at his disposal. Smith (1960) records an examination of a number of dispensing balances and the errors involved in their use He makes a plea for the production of a fine dispensing balance capable of weighings of 1 to 10 grains with an accuracy of  $\pm 1$  per cent This would obviate the need for the use of a fine chemical balance generally recommended for weighings of small quantities of potent substances He doubts that, from his observations, it is worth while using a chemical balance for such weighings and recommends that they be carried out with special care on an ordinary dispensing balance in a draught-free atmosphere on a level bench, having ensured that the balance is regularly cleaned and serviced It is sound practice never to attempt to weigh less than 1 grain or 50 milligrams Smaller quantities can always be obtained by making dilutions and taking an aliquot portion

Losses occur when a weighed solid is transferred by its adherence to the scale pan If the solid is first transferred to paper a further loss may occur The extent of such losses depend on the physical nature of the solid and the surfaces involved If the solid is to be dissolved it may be washed from the scale pan with little or no loss Capper and Dare (1957) recorded the losses on scale pan and paper of a number of chemicals For most substances the loss recorded was small (less than 0.1 per cent) but an exception was a total loss of 15 per cent in weighing 1 grain of calomel and transferring it by means of paper

**Measuring** The student will find it instructive to read the report 'Dispensing Tolerances in Liquid Medicines', Capper and Dare (1957) This report records the variation in accuracy, in measuring fluid medicines by three procedures— By summing the various errors involved in individual operations

By laboratory experiment  
By measurements in actual practice

Some of these points are discussed below

**Standard Measures** Government stamped measures must be used in dispensing The accepted tolerances for these are set out in the British Standard 1921 1953 (Imperial Units) and British Standard 1922 1953 (Metric Units) In measuring, the graduation mark should be aligned with the sighting mark on the back of the measure and with the bottom of the meniscus In addition to the limited errors in the measures an additional error is involved since water is used for graduation and other liquids do not necessarily have a meniscus of the same shape Greasy measures may also be the cause of inaccuracy since the surface tension effect will alter the shape of the meniscus Moreover, the bottom of the meniscus is not always clearly identified This is particularly so with suspensions and other opaque liquids, but sometimes with clear liquids the nature of the lighting and background may cause the appearance of a false meniscus

Some loss is inevitable when a liquid is transferred from a measure to a container or other vessel This will depend on the nature of the liquid Glycerin, for example, will adhere to the glass much more than water Generally this error can be overcome by rinsing out the measure with some of the vehicle, and this must be done whenever possible

Inaccuracy is also possible when adjusting a liquid medicine to its final volume, although in this case the volume is generally considerable and the error less than when measuring small volumes Such adjustments should be carried out in a measure or a carefully calibrated bottle The latter is preferable for suspensions which may be difficult to transfer without loss of sediment

### BIBLIOGRAPHY\*

Before undertaking to dispense a prescription, the student should never hesitate to confirm knowledge or correct guidance For this purpose, and for the wider object of supplementing information on drugs and processes, the following publications will be found useful, but by no means exhaustive

*British Pharmacopœia*, 1963 (Pharmaceutical Press, London)

*British Pharmaceutical Codex*, 1963 (Pharmaceutical Press, London)

*Extra Pharmacopœia* Martindale, 2 vols (Pharmaceutical Press London)

---

\* Useful information has been obtained from some of the books listed and is hereby acknowledged.

## Typical Textbook Entry (Left-hand page)

**EXERCISE 1.1\****Recipe*

Extractum Hamamelidis Sicci	grana tria
Olei Theobromatis	quantum sufficiat
Fiat suppositorium	Mitte sex
Signatur: Unum ut dicto utendum.	

*Translation*

Take	Quantities For 8
Hamamelis Dry Extract	3 grains
Theobroma Oil	24 grains
Make a suppository	a sufficient quantity
Label One to be used as directed	Send six
	104 grains

*Type*

Suppository containing an insoluble solid

*Calculation*

The displacement value of Hamamelis Dry Extract with respect to Theobroma Oil is 1.5, therefore, 24 grains will displace  $24/1.5 = 16$  grains of the oil. The quantity of Theobroma Oil required is therefore,  $120 - 16 = 104$  grains

*Method*

- 1 Wrote the label
- 2 Lubricated the mould
- 3 Placed the weighed quantity of cocoa butter, suitably shredded, in a small evaporating dish. Warmed over a water bath until about two thirds was melted. Removed from the water bath and stirred with a spatula until all the oil was melted. This method avoided overheating
- 4 The extract was placed on a slightly warmed slab and levigated with about half the melted oil until quite smooth. This mix was returned to the evaporating dish and stirred until the whole was quite homogeneous and just too thick to pour. Rewarmed over the water bath until just pourable
- 5 Poured into the lubricated mould, slightly overfilling each cavity
- 6 When set, the excess was trimmed off with a sharp knife
- 7 The mould was set aside until the mass had set hard
- 8 Opened the mould and removed the suppositories
- 9 Washed the suppositories free from lubricant by dropping them into cold water, removing and drying on a perfectly clean cloth
- 10 Placed the suppositories in a divided box lined with waxed paper
- 11 Checked the label, affixed it to the box and wrapped the product

When dispensing a similar type of preparation later in the course, or when repeating an exercise, the report can be considerably curtailed provided all necessary quantities etc. are clearly stated.

*Pharmacopoeia of the United States XVI*  
*Pharmacopoeia Internationalis*, First Edition, Vols  
 I and II and Supplement  
*Textbook of Pharmaceutics*, Bentley (Balliere,  
 Tindall & Cox)  
*Practice of Pharmacy*, Remington (Lippincott,  
 Philadelphia, Pitman Medical, London)  
*United States Dispensatory* (Lippincott, Phila-  
 delphia, Pitman Medical London)  
*The Art of Dispensing* (Chemist & Druggist,  
 London)  
*Latin for Pharmaceutical Students*, Cooper &  
 McLaren (Pitman Medical London)  
*Tutorial Pharmacy*, Cooper & Gunn (Pitman  
 Medical, London)  
*The Pharmaceutical Pocket Book*, 17th Edn  
 (Pharmaceutical Press, London)

## Periodicals

*Pharmaceutical Journal*  
*Chemist & Druggist*

*Journal of Pharmacy & Pharmacology*  
*Journal of the American Pharmaceutical Associa-  
 tion* (Practical Pharmacy Edition.)  
*Journal of Pharmaceutical Sciences*  
*Pharmacy Digest*  
*Public Pharmacist*

## REFERENCES

- CAPPER, K. R., COWELL, D. B. and THOMAS, J. A. (1955) The Measurement of Liquids *Pharm J* 175, 241-246  
 CAPPER, K. R. and DARE, J. G. (1957) Dispensing Tolerances in Liquid Medicines *ibid.* 179, 231-239  
 PARKINSON, J. C. and ROGERS, A. R. (1956) Errors in the Measurement of Water *ibid.* 176, 382  
 SMITH, G. (1960) Examination of a Number of Dispensing Balances *ibid* 185, 473-478



## Weights and Measures

### Rationalisation

ALTHOUGH pharmacists seem to be able to cope remarkably well with two systems of weights and measures it cannot be denied that it would be more convenient if only one were used. The tendency to favour a decimal system has been apparent for many years and is certain to be adopted eventually, and exclusively, in medicine and pharmacy. This being so, pharmacists have been anxious that the details of the transition shall be satisfactory to them and have accordingly been active in negotiations on the subject. The following details give an outline of changes that have taken place already and proposals for the future.

### *The British Pharmacopoeia and the Metric System*

Metric units were introduced into the B.P. 1898 as an alternative to Imperial units in formulae. Doses were expressed only in apothecaries' units.

The British Pharmacopoeias of 1914, 1932, and 1948 gave formulae in metric units only. Doses were given in metric units with equivalents in the apothecaries' system.

The B.P. 1953 followed the above method but omitted apothecary units for drugs normally prescribed in the metric system. These included most of the newly introduced drugs.

The tendency was extended in the B.P. 1958 by further curtailing the use of the apothecaries' system for directed dosage to be given when no dose is prescribed. Only metric units are employed in the B.P. 1963.

### *The British Pharmaceutical Codex and the British National Formulary*

Formulae in these books are given in both systems. A change to the exclusive use of the metric system in the British National Formulary would have much

greater impact on extemporaneous dispensing than a similar change in the *British Pharmacopoeia* and some details of this are considered later.

### *Review of Weights and Measures Legislation*

The President of the Board of Trade (1948) appointed a committee to investigate the legislation relating to weights and measures with the intention of making recommendations which would bring it into line with present-day requirements. Among others the views of the British Medical Association and the Pharmaceutical Society were submitted to the committee. The important recommendation made was—

To abolish the apothecaries system at a specified time in the relatively near future.

It was evident that many difficulties existed and although certain recommendations were made and published in 1951, no legislation resulted from the deliberations.

### *Positive Action by the Association of British Pharmaceutical Industry*

The Association (1952) made recommendations which were carried into effect in 1953, that, in wholesale trading, the metric system of weights and measures should be used in selling pharmaceutical chemicals, preparations etc.

### *Standard Metric Measures*

The British Standards Institution published (1953) standards for metric dispensing measures.

### *Other Recommendations*

The British Association for the Advancement of Science and the Association of British Chambers of Commerce published (1960) a joint report recommending a wider use of the metric system in industry and commerce and indicating that such a change

would have decided advantages if a monetary decimal system were also adopted, and suggested that such a change should be investigated by the Government. This suggestion has now been adopted. The report comments on the proposed abandonment of the apothecaries' system as follows: "The system is being replaced by the metric system voluntarily, slowly but steadily, but while doctors continue to prescribe in apothecaries' units, pharmacists will still have to use them."

#### *Recommendations of the British Pharmacopoeia Commission and Proposed Action*

The General Medical Council accepted a recommendation (1955) of the British Pharmacopoeia Commission to abandon the apothecaries' system in the B.P. 1963. The commission's report further suggested that although difficulties might be experienced in making the change so far as certain liquid medicines are concerned an 'attractive first stage' could be in relation to many official dosage forms such as tablets, capsules and injections.

Capper (1960), at the Pharmaceutical Conference, presented an excellent and comprehensive survey on the position as it has developed, the difficulties involved in changing to the exclusive use of the metric system, and the possible stages by which the change could be achieved. The proposals for the changes are summarised as follows—

*First Stage* All tablets, capsules, injections to be in metric doses. This has already been decided for the B.P. 1963 and there seems little reason for not doing the same for the relatively small number of B.P.C. and B.N.F. preparations of this type.

*Second Stage* Ointments, lotions, eye-drops and other external preparations to be dispensed only in metric quantities. The time for this change would be determined by agreement with the medical profession and the Ministry of Health and these preparations would then be supplied in metric quantities however prescribed.

*Third Stage* Mixtures, linctuses and other liquid internal medicines usually dispensed extemporaneously. This would mark the complete abolition of the apothecaries' system and should occur only when we can be assured that most medical practitioners wish to prescribe in the metric system and are prepared to try to do so. If, for instance, the prescriber wishes to add another medicament to a B.N.F. mixture, he should write this on the prescription in metric units.

To accomplish the change for the *first stage* it will be necessary to stipulate—

- (a) the strength in metric units which *must* be dispensed or supplied in the absence of directions on the prescription or order
- (b) the strength in *metric units* which *must* be dispensed or supplied when a strength in apothecaries' units is stated on the prescription or order

In the case of (a) there is no particular problem but for (b) legislation will be required so that the pharmacist dispensing the prescription may be protected.

The second stage involves preparations for external use which are commonly prescribed in percentage terms. This invites the use of a decimal system. The implementation of this stage would finally remove the confusion between the Imperial and Apothecaries' ounce in the dispensing of ointments and other solid external preparations.

The third stage is the most difficult to accomplish, and the ease with which the change can be made will depend on the willingness of doctors to prescribe in the metric system. Mixtures are usually prescribed in the apothecaries' system. Some of the problems of changing are as follows—

*Dosage* There is no simple metric equivalent of the teaspoonful or tablespoonful. The teaspoonful is considered to be equivalent to 60 minims. This is 3.5 millilitres, but examples of teaspoon capacities have been shown to vary from 2.4 to 7 millilitres. The tablespoonful is considered to be equivalent to 240 minims. This is 14.2 millilitres but tablespoon capacities may vary from 13.4 to 27.2 millilitres. These variations are reported by Spalton (1949). A tablespoonful is generally accepted as 15 ml. In Britain a teaspoonful is usually accepted as 4 ml. Elsewhere 5 ml is accepted as a suitable equivalent and is a better 'metric' quantity, but very different from 3.5 ml (60 minims).

*Containers* Bottles suitable for quantities in fluid ounces will be incorrect for metric quantities. It is reasonable to expect that medicines should be supplied in full bottles but the provision of a completely new set of bottle sizes is a serious economic problem.

*Standard Formulae* The formulae in the B.N.F. for mixtures are reasonable in only one system and to prepare good metric formulae, agreement on suitable quantities must be accepted by medical men. This is obvious if one examines the following example provided by Dr Capper in his report.

*Alkaline Mixture of Ipecacuanha*

	Present formula	Adjusted formula
Sodium bicarbonate	10 gr = 648 mg	600 mg = 9½ gr
Ammonium bicarbonate	3 gr = 194 mg	200 mg = 3⅓ gr
Ipecacuanha tincture	10 min = 0.59 ml	0.6 ml = 10⅓ min
Chloroform water to	½ fl oz = 14.2 ml	15 ml = 153 min

The report further suggests that a Code of Practice for Prescribing in the Metric System should be prepared by the medical and pharmaceutical professions. Such a code would have to consider the following—

- 1 Methods for writing metric quantities on prescriptions
- 2 A standard range of total volumes for liquid medicines
- 3 Dose volume of liquid medicines
- 4 The range of metric doses for drugs in tablets and capsules

## REFERENCES

- 1 Report of the Committee on Weights and Measures Legislation (1951) H M S O Cmd 8219
- 2 A B P I Committee's Report (1952) Use of the Metric System *Pharm J* 168, 284
- 3 British Standards 1922-1953 Dispensing Measures for Pharmaceutical Purposes (Metric Units)
- 4 Joint Report of the British Association for the Advancement of Science and The Association of British Chambers of Commerce (1960) *Decimal Coinage and the Metric System, Should Britain Change?* Butterworth Scientific, London
- 5 Report of the Pharmacopoeia Committee of the General Medical Council (1955) on the proposed abandonment of the apothecaries system in the B P 1963 *Pharm J* 174, 423
- 6 CAPPER, K R (1960) The Change to the Metric System in Pharmaceutical Practice *Pharm J* 185, 229
- 7 SPALTON, L M (1949) Teaspoons and Tablespoons *Pharm J* 162, 338

## THE IMPERIAL SYSTEM

## MEASURES OF MASS (WEIGHT)

All measures of mass are derived from the Imperial Standard Pound. This is cylindrical, being about 1.15 in in diameter and about 1.35 in thick, with an encircling groove or channel about 1 in from its under surface, into which fits the ivory fork used to lift the cylinder. The latter is made of platinum and bears the inscription PS 1884, 1 lb. This cylinder was adopted as the Imperial Standard Pound by the Weights and Measures Act, 1878, 1 lb being defined in that Act as the weight of the cylinder *in vacuo*.

The Imperial Standard Pound is kept at the Standards Dept of the Board of Trade in care of the Warden of the Standards. To cover possible loss of or damage to the original standard an exact duplicate is lodged with each of the following bodies: The Royal Mint, The Royal Observatory, Greenwich, The Royal Society of London, and The Houses of Parliament.

Perfect copies of the Imperial Standard Pound for use in routine testing are known as Board of Trade Standards and are used by local authorities and other bodies charged with the duty of ensuring that all weights used in commerce conform to specification.

All other measures of mass are derived from the Imperial Standard Pound, thus—

1/16th part of Imperial Standard lb is 1 oz avoirdupois  
1/7,000th part of Imperial Standard lb is 1 gr.  
It follows from this that 1 oz avoirdupois contains  
 $\frac{7,000}{16} = 437.5$  grains

The Imperial System includes a set of special weights known as Apothecaries or Troy Weights. These are based on the grain (as defined above) and are as follows

20 grains form	1 scruple
60 " "	1 drachm
480 " "	1 Apothecaries or Troy ounce
12 Apothecaries or Troy ounces form	1 Apothecaries or Troy pound

The following points should be noticed—

1 The grain is the same in both Avoirdupois and Apothecaries' Systems

2 There is no counterpart in the Avoirdupois System to the scruple of the Apothecaries' System, hence confusion cannot occur

3 Care must be taken not to shorten the word drachm to dram. The Avoirdupois System includes a weight called the dram, which is  $\frac{1}{16}$  of the Avoirdupois ounce, and therefore contains  $\frac{437.5}{16} =$

27 34375 grains, whereas the drachm contains 60 grains

4 Care must be taken to distinguish the Apothecaries ounce and the Avoirdupois ounce. The following convention is widely accepted—

The word 'ounce' or its abbreviation 'oz' without qualification means an avoirdupois ounce.

The symbol ʒ means an Apothecaries or Troy ounce, when written at length it must therefore be translated as '1 oz Troy or Apothecaries'—the former expression being usual.

The above convention applies throughout this book.

5 The Apothecaries or Troy pound is not used in Pharmacy and may be disregarded.

#### IMPERIAL WEIGHTS

The Imperial weights in daily use therefore include—

(a) *A set of Grain Weights*, which comprises  $\frac{1}{2}$  grain, 1 grain, 2 grains, 3 grains, 4 grains, 5 grains, and 6 grains. They are cut from thin sheet metal, and the denomination is stamped on each.

(b) *A set of 'Drachm' Weights*, comprising—

$\frac{1}{2}$ scruple	representing	10 grains
1 "	"	20 "
$\frac{1}{2}$ drachm	"	30 "
2 scruples	"	40 "
1 drachm	"	60 "
2 drachms	"	120 "

This set is made in brass, in the form of flat circular discs or squares.

(c) *A set of Apothecaries or Troy Ounce Weights*, usually including 1, 2, 3, 5, 8, 10 and 20 Apothecaries or Troy ounce. The 1 ounce is usually flat and square, but the others are cup-shaped for nesting.

This distinctive shape also distinguishes them from avoirdupois weights, which are flat.

This set is needed when quantities outside the range of the grain or drachm sets are required, and Apothecaries or Troy ounces are specified.

(d) *A set of Avoirdupois Weights*, usually including  $\frac{1}{2}$ , 1, 2, 4, 8 and 16 oz. These are in a flat, cylindrical form, recessed on the upper surface to accommodate the next smaller size—the complete set forming a cylindrical pyramid.

This set is marked Avoirdupois, and is used in commercial transactions where "ounces" without further specification, or with the word avoirdupois, occurs.

#### SYMBOLS USED FOR APOTHECARIES WEIGHTS

In prescriptions the denominations, grain, scruple, drachm, and Troy or Apothecaries ounce are represented by symbols—

grain . . . .	gr	drachm . . . .	3
scruple . . . .	ʒ	Troy ounce . . . .	ʒ

The following points should be noticed—

- 1 The letter 'g' must not be used for grain—it is possible to confuse it with g, used in all scientific and engineering books, for gramme, but not in this book (see note on p. 13).
- 2 The symbol for the scruple consists of a half-circle with a line drawn through the mid point, this line being continued to form the numeral behind the symbol, thus ʒ.
- 3 The drachm symbol resembles figure 3, and is readily distinguished from the symbol for the scruple by the 'break in its back,' formed where the two half-circles join.
- 4 The symbol for the Apothecaries or Troy ounce is made by adding an extra half-circle to the symbol for drachm.

Arabic numerals are used in conjunction with the English words grain, scruple, drachm, ounce (either avoirdupois or apothecaries), and are placed before the word, thus—6 grains, 2 drachms, 3 oz (Troy).

Roman numerals are used in conjunction with the symbols gr, ʒ, ʒ, ʒ, ʒ (and with other symbols), and are placed after the symbol. For example—

ʒ i	1 scruple or 20 grains
ʒ iv	4 drachms
ʒ iii	3 Apothecaries or Troy ounces

Table 21  
Measures of Mass (Weight)

Latin name	Symbol	English name	Equivalent (grains)
Granum	gr	grain	1 grain
Scrupulus	ʒ	scruple	20 "
Drachma	ʒ	drachm*	60 "
Uncia	oz	ounce	437.5 "
		(Avoirdupois)	
Uncia	ʒ	ounce (Troy or Apothecaries)	480 "
Libra	Ib	pound (Troy or Apothecaries)	5,760 "
Libra	Ib	pound (Avoirdupois)	7,000 "

\* Distinguish from "dram," which weighs 27 34375 grains ( $\frac{1}{12}$  of an avoirdupois ounce).

The unit 'one' is often expressed by the letter  $\text{j}$ , when two or more units occur together only the final one is expressed by  $\text{j}$ , e.g.  $\frac{3}{2}\text{j}$ ,  $\frac{3}{2}\text{j}$

The placing of Roman numerals after the symbols should be carefully noted, as it is a common error to find students writing  $1\text{j}3$  to express 1 drachm.

Half is expressed by ss, often written fs (derived from Latin *semu*, half) thus—

$\frac{3}{2}\text{ss}$       half a drachm or 30 grains  
 $\frac{3}{2}\text{fs}$       half an Apothecaries ounce or 240 grains

Table 21 summarises the principal measures of mass used in pharmacy, and should be thoroughly mastered without delay

### MEASURES OF CAPACITY (VOLUME)

The unit or standard measure of capacity is the Imperial Standard Gallon, and this is a secondary or derived standard, being the volume occupied by 10 Imperial Standard pounds weight of distilled water, weighted in air, at  $62^{\circ}\text{F}$  and with the barometer at 30 inches. A brass vessel of this capacity was prepared in 1824, and forms the original standard, being marked 'Imperial Standard Gallon, Anno Domini MDCCCXXIV Anno V G IV Regis'. It is in the custody of the Warden of the Standards in the Department of the Board of Trade, and exact copies are used as Board of Trade Standards.

All other measures of capacity are derived from the Imperial Standard Gallon—

$\frac{1}{6}$ part of Imperial Standard Gallon is	1 pint
$\frac{1}{60}$ "      "      "	1 fl oz
$\frac{1}{6}$ "      fluid ounce is	1 fl drachm
$\frac{1}{60}$ "      fluid drachm is	1 minim

The smaller measures of capacity (e.g. up to 1 pint) used in pharmacy are made of glass and are either conical or cylindrical, the larger measures are usually of metal. The graduations on each measure are clearly marked with their denomination, and they must not be nearer each other than  $\frac{1}{12}$  in. Hence a measure holding one ounce or more may not be graduated at every 20 or 30 minims.

The surface of a liquid in a vessel is concave, and the correct reading is taken from the lowermost point of the concave surface, or meniscus.

The distant surface of glass measures bears a number of engraved lines, each corresponding with one of the graduations on the near surface of the measure. These are provided to check levelling of the measure when held in the hand, the measure being level when a graduation, *on a level with the eye*, covers the corresponding line on the back of the

measure. The graduation selected to check levelling may, if the liquid to be measured is colourless, be the volume required, in which case the eye, the graduation, the lowest point of the meniscus, and the line on the back of the measure, must all be in alignment, in other cases a graduation above the volume to be measured serves to check levelling.

### SYMBOLS USED FOR IMPERIAL MEASURES OF CAPACITY

In prescriptions the denominations minim, fluid drachm, and fluid ounce are represented as follows—

minim	m	fluid drachm	.	3
fluid ounce	$\frac{3}{2}$	pint	.	0
gallon	C			

The following points should be noticed—

1 In measures of capacity there is no counterpart to the scruple.

2 The symbols for the fluid drachm and the fluid ounce are identical with those used for drachm and troy ounce for solids by weight. This does not lead to confusion, because it is an unvarying rule in this country to weigh solids and to measure liquids, unless otherwise specified, hence the symbol for drachm used in respect of a solid means 60 grains, used in respect of a liquid it means 60 minims. It follows that the word 'fluid' need not be placed before drachm or ounce, because these denominations will be uniformly interpreted to mean fluid drachms or fluid ounces if the substance is a liquid, and 60 grains and 437 5 grains respectively if the substance is a solid.

3 For solids there are two ounces, the avoirdupois and the troy, rendering careful distinction important, but for liquids there is only one fluid ounce, and confusion is not possible.

As before, arabic numerals are used in conjunction with the English words minim, ounce, pint,

Table 2.2  
Measures of Capacity (Volume)

Latin name	Symbol	English name	Equivalent
Minimum	m	minim	
Gutta	gtt	drop	
Fluidrachma	$\frac{3}{2}$	fl drachm	60 minims
Fluiduncia	3	fl ounce	8 fl drachms
Octarius	0	pint	20 fl ounces
Congius	C	gallon	8 pints

gallon, and are placed before the word roman numerals are used in conjunction with the symbols, and are placed after the symbol.

Table 2.2 summarises the principal measures of capacity used in pharmacy, and should be thoroughly mastered at once—

## METRIC SYSTEM

### MEASURES OF MASS (WEIGHT)

The Metric System was legalised for use in this country by the Weights and Measures (Metric System) Act, 1897, and a number of metric standards were defined and adopted

Table 2.3  
Measures of Mass (Weight)

<i>Latin name</i>	<i>Abbreviation</i>	<i>English name (gramme)</i>	<i>Equivalent</i>
Micro-gramma	$\mu\text{g}^*$	micro-gramme	$1 \times 10^{-6}$
Milligramma	mg	milligramme	0.001
Gramma	g or G†	gramme	1.0
Kilogramma	kg	kilogramme	1,000

\* The B.P. recommends that in writing prescriptions and expressing doses 'microgram' should be written in full and when an abbreviation is essential 'mcg' should be used

† The B.P., B.P.C. and B.N.F. recommend the use of the symbol G for gramme in writing prescriptions and stating doses. This is a departure from international practice but is necessary to avoid confusion between gramme and grain. The symbol g for gramme is not confusing to practitioners but students are advised always to use the symbol G, which is used throughout this book

Table 2.4  
Measures of Capacity (Volume)

The primary standard for measures of capacity in the metric system is the litre

<i>Latin name</i>	<i>Abbreviation</i>	<i>English name (millilitre)</i>	<i>Equivalent</i>
Millilitra	ml†	millilitre	1.0
Litra	l	litre	1,000

† The cubic centimetre (c.c.) is slightly less than the ml 1 ml being equal to 1.000028 c.c., but they are regarded as identical for all practical purposes.

The primary standard for measures of mass in the metric system, i.e. the standard from which all other measures of mass are derived, is the kilogramme. Special names are given to sub-multiples of the kilogramme, thus  $10^{-6}$  part is 1 gramme. Tables 2.3 and 2.4 should now be mastered.

The Pharmacopoeia uses the metric system exclusively

### METRIC AND IMPERIAL QUANTITIES

As mentioned, the Pharmacopoeia uses only the metric system in formulae, but prescriptions are commonly written in the Imperial system

The following will serve whenever it is necessary to make a pharmacopoeial preparation to fill a prescription in the Imperial system—the metric quantity being slightly larger than the Imperial—

30 ml for 1 fluid ounce  
30 G for 1 ounce avoirdupois\*

For fluid preparations the pharmacopoeial formula usually produces 1,000 ml (35.2 ounces approx.), and to obtain round numbers it is sometimes necessary to prepare—

100 ml for 3 fluid ounces  
120 ml for 4 fluid ounces  
200 ml for 6 fluid ounces  
240 ml for 8 fluid ounces

\* 30 G is insufficient for 1 ounce troy (= 31 grammes approx.), hence the nearest convenient quantity above 31 G should be prepared.

## 3

## Calculations

## PERCENTAGE CALCULATIONS\*

## Percentage Solutions of Solids in Liquids

THERE are two kinds of percentage solutions of solids in liquids—

(a) Weight in volume (*w/v*) solutions

The general formula for these is—

Solid                    1 part by weight } for 1%  
Solvent to produce 100 parts by volume } w/v

This kind is common in pharmacy and is always supplied when *w/w* is not specially requested

(b) Weight in weight (*w/w*) solutions

The general formula for these is—

Solid                    1 part by weight } for 1%  
Solvent to produce 100 parts by weight } w/w

This kind is rarely required in pharmacy, and is not used unless specially requested

It is discussed on p 28

## WEIGHT IN VOLUME PERCENTAGE SOLUTIONS

In transposing the general formula—

Solid                    1 part by weight } for 1%  
Solvent to produce 100 parts by volume } w/v

the measure of mass used for the 'part by weight' must be strictly comparable (i.e. identical when water is used for comparison) with the measure of capacity used for the 'parts by volume'

In the metric system the gramme as a measure of mass is strictly comparable with the millilitre as a measure of capacity (i.e. 1 G of water measures 1 ml), hence in the metric system the above general formula becomes—

Solid                    1 G  
Solvent to produce 100 ml } for 1% w/v

Calculations for *w/v* percentage solutions in the metric system are based on the above (Example 3 1)

The relationships between measures of mass and measures of capacity in the Imperial system are shown in Table 3 I

## EXAMPLE 3 1

Prepare 200 ml of a 5 per cent solution of sodium chloride  
*Calculation*—

$$\frac{1 \text{ G with solvent to produce } 100 \text{ ml} = 1\% \text{ w/v}}{1 \times 5 \times 200} \quad \text{“ “ } 200 \text{ ml} = 5\% \text{ w/v}$$

$$\frac{100}{= 10 \text{ G}}$$

10 G of sodium chloride is therefore dissolved in sufficient water to produce 200 ml of solution

\* The student should refer to the definitions of percentages in the General Notices on page 7 of the *British Pharmacopoeia 1963*

Table 31

<i>Derived standards of mass</i>	<i>Primary standard from which other measures of mass and capacity are derived</i>	<i>Derived standards of capacity</i>
	10 lb of water constitute 1 gallon	10 lb of water constitute 1 gallon i.e. the volume occupied by 160 oz avoirdupois of water constitutes 1 gallon
Imperial Standard Pound	½ of a gallon constitutes 1 pint	½ of a gallon constitutes 1 pint i.e. the volume occupied by 20 oz avoirdupois of water constitutes 1 pint
1/16 of a pound constitutes 1 oz avoirdupois	½ of a pint constitutes 1 fluid oz	½ of a pint constitutes 1 fluid oz i.e. the volume occupied by 1 oz avoirdupois of water constitutes 1 fluid oz

In the Imperial system the oz avoirdupois as a measure of mass is therefore strictly comparable with the fluid ounce as a measure of capacity (i.e. 1 oz avoirdupois of water measures 1 fluid oz). Hence in the Imperial system the general formula becomes—

$$\text{Solid } \frac{1}{16} \text{ oz avoirdupois} \quad \text{Solvent to produce 100 fluid oz} \quad \left\{ \text{for 1\% w/v} \right.$$

The grain is a derived standard and is  $\frac{1}{7,000}$  th part of the standard pound, hence the oz avoirdupois contains  $\frac{7,000}{16} = 437.5$  gr.

The above formula, written for 1 fl. oz of solution, therefore becomes

$$\text{Solid } \frac{1}{100} \text{ oz avoirdupois} = \frac{437.5}{100} \text{ gr} = 4.375 \text{ gr} \quad \left\{ \text{for 1\% w/v} \right. \\ \text{Solvent} \quad \text{to produce 1 fluid oz} \quad \left. \right\}$$

The minum is a derived standard and is  $\frac{1}{480}$  th part of the fluid ounce, hence the above formula may be written—

$$\text{Solid } 4.375 \text{ gr} \quad \left\{ \text{for 1\% w/v} \right. \\ \text{Solvent} \quad \text{to produce 480 minums} \quad \left. \right\}$$

It follows, therefore, that a 1% w/v solution contains—

$$1 \text{ grain of solid in } \frac{480}{4375} \text{ minums} = 109.7 \text{ minums}$$

The figure 109.7 is rounded off to 110, and a 1 per cent w/v solution is for ordinary purposes, regarded as containing 1 gr in 110 minums.

It must be carefully noted that 1 gr in 100 minums is not a 1 per cent w/v solution, owing to the fact that

the grain as a measure of mass is not quite comparable with the minum as a measure of volume, thus—

$$\begin{array}{ll} \text{1 oz avoirdupois} & \text{is strictly comparable with 1 fluid oz.} \\ \text{A grain is} & \text{A minum is} \\ \frac{1}{437.5} & \frac{1}{480} \\ \text{part of this} & \text{part of this} \end{array}$$

Consequently a grain is not strictly comparable with a minum.

Solutions made to the following formula are therefore identical in strength—

$$\begin{array}{lllll} \text{Solid} & 1 \text{ G} & 1 \text{ oz} & 4.375 & 1 \\ & & \text{avoirdupois} & \text{gr} & \text{gr} \\ \text{Solvent} & 100 & 100 & 1 & 110 \\ \text{to produce} & \text{ml} & \text{fl. oz} & \text{fl. oz} & \text{m} \end{array}$$

#### TRANSPOSITION FROM METRIC TO IMPERIAL QUANTITIES

The Pharmacopoeia states formulae in the metric system only, and there are occasions when it is convenient to use Imperial weights and measures. Wherever possible the student is recommended to use the metric system. This is so even when a quantity is demanded in the Imperial System. A suitable excess can usually be made using convenient metric quantities. See p. 13

The relationships established above show that the following rules for transposition are applicable—

**Rule 1** G may be read as oz avoirdupois  
Ml may be read as fluid oz.

**Rule 2** G may be read as grains  
Ml may be read as minums, after adding 10 per cent to the figure

Rule 1 provides a basis for large batches  
 Rule 2 is for small batches, and a suitable multiple or sub-multiple is used when necessary  
 Examples 3 2 and 3 3 illustrate the application of the rule of transposition

#### PREPARING FRACTIONAL QUANTITIES FROM A FORMULA

When preparing a fractional quantity of an official preparation care should be taken to check the figures by adding the total quantity of the reduced formula or by comparing the quantities one with another. Examples 3 4 and 3 5 serve to illustrate this.

#### CALCULATION OF PERCENTAGE SOLUTIONS (w/v) IN IMPERIAL SYSTEM

There are three convenient starting points for these calculations—

Method 1	Solid	1 gr	for 1% w/v solution
	Solvent, to produce	110 minums	
Method 2	Solid	4 375 gr	
	Solvent, to produce	1 fluid oz	Method 3
Method 3	Solid	35 gr	
	Solvent, to produce	8 fluid oz	

Method 1 should be used when the volume of the solution required is small, and the strength of the solution required is weak.

Method 2 or 3 should be used in other instances, for preference No 3 (which is eight times No 2), because the arithmetic is usually simpler.

#### Examples of Methods 1 and 2

In applying Method 1 it is important to note that it is not permitted to weigh less than 1 gr of solid, hence the volume prepared may greatly exceed that required (See Examples 3 6, 3 7 and 3 8)

#### EXAMPLE 3 2

State the formula for 20 fluid ounces of Oily Lotion of Calamine. The batch is fairly large and, therefore, Rule 1 forms the basis for transposition. The quantities obtained in this way produce 1,000 fluid ounces of lotion, hence the submultiple required for 20 fluid ounces is 20/1,000ths or 2/100ths of the transposed quantities thus—

Formula for Oily Lotion of Calamine	Transposing (Rule 1)	2/100ths of Transposed Quantities
Calamine	50 G	50 oz
Wool Fat	10 G	10 oz
Arachus Oil	500 ml	500 fl oz
Oleic Acid	5 ml	5 fl oz
Calcium Hydroxide Solution	1,000 ml to 1,000 fl oz	to 20 fl oz

#### EXAMPLE 3 3

State the formula for 1 pint of Soap Liniment

##### Calculation—

Again the batch is fairly large and therefore Rule 1 forms the basis for transposition, the quantities obtained in this way produce 1,000 fluid oz of liniment, hence  $\frac{1}{1000}$ th =  $\frac{1}{50}$ th of the transposed quantities is the required sub-multiple

(continued overleaf)

*Example 3.3 continued*

<i>Formula for Liniment of Soap</i>	<i>Transposing (Rule 1)</i>	$\frac{1}{50}$ of Transposed Quantities
Camphor	40 G	40 oz
Oleic Acid	40 G	40 oz
Alcohol (90 per cent)	700 ml	700 fl oz
Potassium Hydroxide Solution	140 ml	140 fl oz
		$\frac{1}{5}$ fl oz = 2 fl. oz 384 minims
Rosemary Oil	15 ml	15 fl oz
Purified Water	to 1,000 ml	to 1,000 fl oz to 20 fl oz

**EXAMPLE 3.4**

Calculate the formula for 60 grammes of Wool Alcohols Ointment

	<i>Official quantities</i>	$\frac{6}{100} \times \text{official quantities}$
Wool Alcohols	60 G	3 6 G
Hard Paraffin	240 G	14 4 G
White or Yellow Soft Paraffin	100 G	6 0 G
Liquid Paraffin	600 G	36 0 G
	1,000 G	60·0 G

This is obviously an excellent check to prevent slips in transposing quantities

**EXAMPLE 3.5**

Calculate the formula for 240 millilitres of Oily Lotion of Calamine

	<i>B.P.C. quantities</i>	$\frac{24}{100} \times \text{B.P.C. quantities}$
Calamine	50 G	12·0 G
Wool Fat	10 G	2·4 G
Oleic Acid	5 ml	1·2 ml
Arachis Oil	500 ml	120·0 ml
Calcium Hydroxide Solution	to 1,000 ml	to 240·0 ml

Although this cannot be checked by adding, the figures can be compared. For example, 50 is to 10 as 12 is to 2 4, 10 is to 5 as 2 4 is to 1 2, 5 is to 500 as 1 2 is to 120

**EXERCISE 3.1**

1. State the formula for—

- (a) 1 quart of Ammoniated Liniment of Camphor
- (b)  $\frac{1}{2}$  pint of Concentrated Peppermint Water

2. State a formula (Imperial system) suitable for—

- (a) 20 fluid oz of Calamine Lotion.
- (b) 2 oz of Wool Alcohols Ointment.

(Answers to this and subsequent Exercises are provided on pp. 592-3)

## EXAMPLE 3 6

*Recipe—*

Atropinæ Sulphatis	$\frac{1}{2}\%$
Aquam	ad $\frac{3}{5}$ fl
Fiant guttae pro oculo dextro	

*Calculation—*

1 gr in 110 minimis is  $\frac{1}{2}\%$  w/v

1 gr in 220 ,  $\frac{1}{2}\%$  w/v

This quantity would be prepared, 2 drachms dispensed, and the remainder rejected

Compare Method 1, above, with the following, Method 2—

4 375 gr in 1 fluid oz is  $\frac{1}{2}\%$  w/v

$\frac{4 375}{4 \times 2}$  gr in  $\frac{1}{2}$  fluid oz is  $\frac{1}{2}\%$  w/v

$$= 0.546875 \text{ gr}$$

This quantity cannot be directly weighed because it is less than 1 gr. It may not be rounded off to 0.5 gr because it would be deficient to approximately 9 per cent. It may, however, be obtained by preparing a solution triturate and using an aliquot portion, but this would waste time without saving material. Method 1 is therefore simpler and not more wasteful than Method 2.

## EXAMPLE 3 7

*Recipe—*

Cocainæ Hydrochloridi	$\frac{2}{2}\%$
Aquam	ad $\frac{3}{4}$ fl
Fiat solutio Sig Guttæ pro oculis	

*Calculation—*

1 gr in 110 minimis is  $\frac{1}{2}\%$  w/v

2 gr in 110 ,  $\frac{2}{2}\%$  w/v

Twice this quantity is not sufficient, three times this quantity is unnecessarily wasteful. Prepare—

$\frac{3}{2}$  times the quantity, namely, 5 gr dissolved in sufficient water to produce 275 minimis

From this amount 4 drachms would be dispensed and the remainder rejected

Compare Method 1, above, with Method 2—

4 375 gr in 1 fluid oz is  $\frac{1}{2}\%$  w/v

$\frac{4 375 \times 2}{2}$  gr in  $\frac{1}{2}$  fluid oz is  $\frac{2}{2}\%$  w/v

$$= 4 375 \text{ gr}$$

This quantity cannot be weighed directly, and the error involved in rounding off to 4.5 gr is almost 3 per cent in excess, and is not justified. The former method is easy and accurate

As an alternative, and with advantage, the metric system may be employed. A suitable quantity would be 15 ml e.g.

Cocaine Hydrochloride 0.3 G

Water to 15.0 ml

Four drachms would be dispensed

**EXAMPLE 38**

Send 1 oz of a  $\frac{1}{2}$  per cent solution of Zinc Sulphate  
Label The Eye Drops

*Calculation—*

$$\begin{array}{l} 1 \text{ gr in } 110 \text{ minims is } 1\% \text{ w/v} \\ 1 \text{ gr in } 880 \quad " \quad \frac{1}{8}\% \text{ w/v} \end{array}$$

This quantity would be prepared, 1 oz dispensed, and the remainder rejected.

Again, compare this with Method 2, thus—

$$\begin{array}{r} 4375 \text{ gr in } 1 \text{ fluid oz is } 1\% \text{ w/v} \\ 4375 \\ \hline g \text{ gr in } 1 \text{ fluid oz is } \frac{1}{8}\% \text{ w/v} \\ = 0.546875 \text{ gr} \end{array}$$

The criticism made under Example 37 applies

**Examples with Two or More Medicaments**

The solution required may contain two or more medicaments. In these cases, waste may often be prevented by making *separate* solutions of each

constituent at double strength (when the solution contains two constituents), at triple strength (when the solution contains three constituents), and so on, and then mixing equal volumes of these solutions (See Example 39)

**EXAMPLE 39**

*Recipe—*

Physostigmine Salicylate	$\frac{1}{2}\%$
Hyoscine Hydrobromide	1%

Fiat solutio Mitte 3 iv Sig Guttae pro oculis

*Calculation—*

To prepare the physostigmine salicylate solution at double strength, i.e.  $\frac{1}{2}$  per cent—

$$\begin{array}{l} 1 \text{ gr in } 110 \text{ minims is } 1\% \text{ w/v} \\ 1 \text{ gr in } 220 \quad " \quad \frac{1}{2}\% \text{ w/v} \end{array}$$

This quantity would be prepared

To prepare the hyoscine hydrobromide solution at double strength, i.e. 2 per cent—

$$\begin{array}{l} 1 \text{ gr in } 110 \text{ minims is } 1\% \text{ w/v} \\ 2 \text{ gr in } 110 \quad " \quad 2\% \text{ w/v} \end{array}$$

This will be insufficient (120 minims is required) and therefore it is necessary to make  $1\frac{1}{2}$  times the quantity, i.e. 3 gr in 165 minims

Mix 120 minims of the physostigmine salicylate solution with 120 minims of the hyoscine hydrobromide solution.

Compare the above with direct preparation of a solution of both constituents—

$$\begin{array}{l} 1 \text{ gr Physostigmine Salicylate in } 110 \text{ minims is } 1\% \text{ w/v} \\ 1 \text{ gr } " \quad " \quad 440 \quad " \quad \frac{1}{2}\% \text{ w/v} \end{array}$$

This is the minimum quantity which may be prepared, and the quantity of hyoscine hydrobromide must therefore be that needed for 440 minims—

$$\begin{array}{l} 1 \text{ gr Hyoscine Hydrobromide in } 110 \text{ minims is } 1\% \text{ w/v} \\ 4 \text{ gr } " \quad " \quad 440 \text{ minims is } 1\% \text{ w/v} \end{array}$$

Preparation in this way, although correct, is needlessly wasteful,

1 gr of hyoscine hydrobromide is saved by adopting the former method. With advantage a quantity could be made in the metric system. A suitable amount would be 20 ml. It is simple to rewrite the formula for 100 ml as follows and deduce the quantities for 20 ml

	<i>Quantities for 20 ml</i>	
Physostigmine Salicylate	250 mg	50 mg
Hyoscine Hydrobromide	1,000 mg	200 mg
Water to	100 ml	to 20 ml

### Examples with Special Solvents

The solvent may consist partly or entirely of an expensive liquid. In these cases, waste may often be prevented thus—

- (a) when the expensive liquid forms only part of the solvent, prepare the solution at double, or higher strength in the cheap solvent, and dilute suitably, including the expensive solvent when diluting
- (b) when the whole of the solvent consists of an expensive liquid, prepare at double (or higher) strength and dilute to requirements  
(Example 3 10)

### Examples of High Dilutions

Small volumes of very weak solutions may be conveniently prepared by first making a stronger solution and then diluting a suitable volume of this to form the weak solution required  
(See Example 3 11)

### Examples of Calculations: Method 3

As mentioned earlier (p. 17), Method 3 is eight times Method 2, and provides simpler arithmetic. Method 3 is used throughout in Examples 3 12 3 13 and 3 14

### EXAMPLE 3 10

Prepare  $\frac{1}{2}$  oz of a  $\frac{1}{2}$  per cent solution of Zinc Sulphate in equal volumes of Normal Saline Solution and Adrenaline Solution  
Label The Eye Drops

#### *Calculation—*

Adrenaline Solution is relatively expensive, but Normal Saline Solution is inexpensive, therefore prepare the Zinc Sulphate at double strength in the latter

1 gr Zinc Sulphate in 110 minims is 1% w/v  
1 gr " " 440 "  $\frac{1}{2}$ % w/v

This quantity would be prepared using normal saline solution as the solvent, and 120 minims of this solution mixed with 120 minims of Adrenaline Solution

Compare the above with *direct* preparation of the solution with a mixture of both solvents

1 gr Zinc Sulphate in 110 minims is 1% w/v  
1 gr " " 880 "  $\frac{1}{2}$ % w/v

Preparation of this quantity using equal volumes of Normal Saline Solution and Adrenaline Solution would involve the use of 440 minims of the latter, i.e. 320 minims more than is used in the former method

### EXAMPLE 3 11

Prepare 1 oz of a 1 in 10 000 solution of Copper Sulphate

#### *Calculation—*

10 000 is  $100 \times 100$ , and it will therefore be convenient to prepare a 1 in 100 solution and then dilute it 100 times, thus—

(continued overleaf)

**Example 3 11 continued**

1 gr Copper Sulphate in 110 minims is 1% w/v, i.e. 1 in 100  
 Prepare this quantity, dilute 5 minims of it to 500 minims and send the 1 oz requested.

Such quantities are readily obtained in the metric system. For example, 50 mg in 50 ml is a 1 in 1,000 solution. Three ml of this solution diluted to 30 ml will give sufficient for 1 fl. oz of a 1 in 10,000 solution

---

**EXERCISE 3.2**

Give a suitable formula for making each of the following—

1 Atropine Sulphate 1 per cent Send 2 drachms  
 Label The Eye Drops

2 Mercury Oxycyanide 1 in 5,000 Send 1 oz  
 Label The Eye Drops

3 Acriflavine 0.1 per cent in a mixture of equal volumes of alcohol (90%) and water Send 1 oz.  
 Label The Ear Drops

---

**EXAMPLE 3 12**

Required 8 oz of a 5 per cent solution of mercuric chloride

*Calculation—*

35 gr, in 8 oz, are required to make a 1 per cent solution.

$\frac{35 \times 5}{8}$  gr, in 8 oz, are required to make a 5 per cent solution.

$\frac{35 \times 5 \times 8}{8}$  gr, in 8 oz, are required to make a 5 per cent solution

$$= 175 \text{ gr}$$

Therefore, 175 gr of mercuric chloride dissolved in water and diluted to 8 oz will produce a 5 per cent solution

---

**EXAMPLE 3 13**

Required 1 quart of a 1 in 400 solution of potassium permanganate

*Calculation—*

35 gr, in 8 oz, are required to make a 1 per cent (1 in 100) solution.

$\frac{35 \times 100}{400}$  gr, in 8 oz, are required to make a 1 in 400 solution.

$\frac{35 \times 40 \times 100}{400 \times 8}$  gr, in 40 oz, are required to make a 1 in 400 solution.

$$= 43.75 \text{ gr}$$

Therefore, 43.75 gr of potassium permanganate dissolved in water and diluted to 1 quart will produce a 1 in 400 solution.

*Note* In practice, fractions of a grain, in such cases as this, are approximated to the nearest half grain. In the above case, 44 grains would be used. This is only possible where the error introduced is small in comparison with the amount used. If a solution were to be prepared using 1.75 gr, it would not do to take 2 gr as being sufficiently accurate, as the error would be 0.25 gr or 14.3 per cent in excess. In such a case, the fraction may be weighed by dissolving 1 gr in a suitable quantity of water

and then taking the required fraction of this solution. Thus, 2 gr ( $\frac{8}{4}$ ) may be dissolved in 8 drachms and 7 drachms taken (= 1.75 gr) and

diluted to the required volume. It is a matter of discretion for the dispenser to decide whether a fraction should be accurately weighed, or weighed to the nearest half-grain.

Note that in the metric system 1,200 ml could be made and calculated as follows—

$$\frac{1,200}{400} = 3 \text{ G}$$

Therefore, 3 G would be dissolved and made up to 1,200 ml, and a quart provided

---

## EXAMPLE 3 14

Required 1 pint of a 1 in 80 solution of zinc sulphate

*Calculation*—

35 gr, in 8 oz, are required to make a 1 per cent (1 in 100) solution

$$\frac{35 \times 100}{80} \text{ gr, in 8 oz, are required to make a 1 in 80 solution}$$

$$\frac{35 \times 100 \times 20}{80 \times 8} \text{ gr, in 20 oz, are required to make a 1 in 80 solution} \\ = 109.375 \text{ gr (use } 109\frac{1}{2} \text{ gr)}$$

Therefore, 109.5 gr of zinc sulphate dissolved in water and diluted to 1 pint will produce a 1 in 80 solution

In the metric system 600 ml could be made. The calculation would be—

$$\frac{600}{80} = 7.5 \text{ G}$$

7.5 G would be dissolved, adjusted to 600 ml, and 1 pint provided

---

## EXAMPLE 3 15

How many tablets, each containing 8.75 gr of corrosive sublimate will be required to make half a gallon of a 1 in 500 solution?

*Calculation*—

35 gr, in 8 oz, are required to make a 1 per cent (1 in 100) solution

$$\frac{35 \times 100}{500} \text{ gr, in 8 oz, are required to make a 1 in 500 solution}$$

$$\frac{35 \times 100 \times 80}{500 \times 8} \text{ gr, in 80 oz, are required to make a 1 in 500 solution} \\ = 70 \text{ gr}$$

Each tablet contains 8.75 gr, so that the number required is—

$$\frac{70}{8.75} = 8$$

Therefore, 8 tablets each of 8.75 gr will make half a gallon of a 1 in 500 solution

---

Many substances are made in tablet form of convenient strength for making solutions. The number of tablets required to make a quantity of solution is readily calculated as shown in Example 3 15 above

When strengths are stated, not in percentage but as 1 part in a specified volume, e.g. 1 in 1,000, mistakes are often made in conversion to percentage. Inspection of the following simple examples should avoid this

$1 \text{ in } 400 = \frac{100}{400} \text{ per cent}$	$= 0.25 \text{ per cent}$	$1 \text{ in } 2500 = \frac{100}{2500} \text{ per cent}$	$= 0.04 \text{ per cent}$
$1 \text{ in } 700 = \frac{100}{700} \text{ per cent}$	$= 0.143 \text{ per cent}$	$1 \text{ in } 4,000 = \frac{100}{4,000} \text{ per cent}$	$= 0.025 \text{ per cent}$
$1 \text{ in } 800 = \frac{100}{800} \text{ per cent}$	$= 0.125 \text{ per cent}$	$1 \text{ in } 5,000 = \frac{100}{5,000} \text{ per cent}$	$= 0.02 \text{ per cent}$
$1 \text{ in } 1,000 = \frac{100}{1,000} \text{ per cent}$	$= 0.1 \text{ per cent}$	$3 \text{ in } 1,000 = \frac{3 \times 100}{1,000} \text{ per cent}$	$= 0.3 \text{ per cent}$
$1 \text{ in } 2,000 = \frac{100}{2,000} \text{ per cent}$	$= 0.05 \text{ per cent}$	$4 \text{ in } 10,000 = \frac{4 \times 100}{10,000} \text{ per cent}$	$= 0.04 \text{ per cent}$

**EXERCISE 33**

Calculate the following—

- 1 4 oz of a 1 in 2,000 solution
- 2 6 oz of a 3 per cent solution
- 3 2 oz of a 16 per cent solution
- 4 12 oz of a 1 in 40 solution
- 5  $\frac{1}{2}$  gallon of a 1 in 1,000 solution
- 6 1 quart of a 0.2 per cent solution
- 7 1 pint of a 0.01 per cent solution
- 8 8 oz of a 0.625 per cent solution
- 9 12 oz of an  $\frac{1}{2}$  per cent solution
- 10 16 oz of a 0.25 per cent solution
- 11 8 oz of a 1 in 800 solution
- 12 1 pint of normal saline solution

(Normal saline solution is a 0.9 per cent solution of sodium chloride.)

- 13 How many tablets each containing 8.75 gr of mercuric chloride, will be required to make—
  - (a) 1 gallon of a 1 in 1,000 solution
  - (b) 1 quart of a 0.05 per cent solution
  - (c) 1 pint of a 0.2 per cent solution

Examples of Solutions Issued in Concentrated Form being diluted by the patient as directed.  
 Solutions are often dispensed in concentrated form (See Examples 3 16, 3 17 and 3 18 also the example to avoid unnecessary bulk, the concentrated solution in Exercise 3 4 )

**EXAMPLE 3 16**Required 4 oz of a solution so that  $\frac{2}{3}$  teaspoonfuls diluted to a pint will make a 1 in 1,000 solution.*Calculation—*

This is calculated in two stages—

- 1 Find how many grains will be required to make a pint of a 1 in 1,000 solution.
- 2 Every 2 teaspoonfuls of the 4 oz of stronger solution must contain this number of grains. Therefore, the number of grains found above must be multiplied by the number of 2 teaspoonfuls contained in the 4 oz.

Thus—

35 gr, in 8 oz, will make a 1 per cent (1 in 100) solution

$$\frac{35 \times 100}{1,000} \text{ gr, in 8 oz, will make a 1 in 1,000 solution}$$

$$\frac{35 \times 100 \times 20}{1,000 \times 8} \text{ gr, in 20 oz, will make a 1 in 1,000 solution}$$

$$= 8.75 \text{ gr}$$

Therefore, 8.75 gr must be contained in every 2 teaspoonfuls of the solution

In 4 oz there are 32 teaspoonfuls

$$\frac{8.75 \times 32}{2} \text{ gr must be contained in 4 oz of the solution} = 140 \text{ gr}$$

Dissolve 140 gr in water and dilute to 4 oz

For the patient's own use, it is correct to label them without stating the strength or dilution, as—

The Solution Two teaspoonfuls to be diluted to 1 pint

#### EXAMPLE 3 17

Required 12 oz of a solution so that 3 teaspoonfuls to a quart will produce an  $\frac{1}{8}$  per cent solution

*Calculation—*

Proceeding as before—

35 gr, in 8 oz, will make a 1 per cent solution

$$\frac{35}{8} \text{ gr, in 8 oz, will make a } \frac{1}{8} \text{ per cent solution}$$

$$\frac{35 \times 40}{8 \times 8} \text{ gr, in 40 oz, will make a } \frac{1}{8} \text{ per cent solution} = 21.875 \text{ gr}$$

Therefore, 21.875 gr must be contained in every 3 teaspoonfuls

In 12 oz there are 96 teaspoonfuls

$$\frac{21.875 \times 96}{3} \text{ gr must be contained in the 12 oz} = 700 \text{ gr}$$

#### EXAMPLE 3 18

Required, 8 oz of a solution so that 1 tablespoonful to half a gallon makes a 1 in 500 solution

*Proceeding as before—*

35 gr, in 8 oz, will make a 1 per cent (1 in 100) solution

$$\frac{35 \times 100}{500} \text{ gr, in 8 oz, will make a 1 in 500 solution}$$

$$\frac{35 \times 100 \times 80}{500 \times 8} \text{ gr, in 80 oz, will make a 1 in 500 solution}$$

$$= 70 \text{ gr}$$

Therefore 70 gr must be contained in every tablespoonful.

In 8 oz there are 16 tablespoonfuls

$$70 \times 16 \text{ gr must be contained in 8 oz} = 1,120 \text{ gr}$$

#### Alternative Methods

The student is advised to study the two following methods which are simple, and which act as a check

upon each other. The methods are based on (a) the given volume of concentrated solution, or (b) the total volume of diluted solution it can make

In each case the calculation resolves itself into—

$\frac{1}{4}$  gr (1% of 1 fl oz)  $\times$  the number of ounces  $\times$  the percentage w/v = grains

Using Example 3.18,

Method (a)

$$\frac{35}{8} \times 8 \times \text{percentage of concentrated solution} = \text{grains}$$

The percentage of concentrated solution can be found by inspection. Since the diluted solution is 1 in 500, and since this is prepared by diluting the concentrated solution 160 times ( $\frac{1}{8}$  fl oz to 80 fl. oz), the concentrated solution must be 160 in 500 or

$$\frac{160 \times 100}{500} \text{ per cent}$$

The calculation, therefore, becomes—

$$\frac{35 \times 8 \times 160 \times 100}{8 \times 500} = 1,120 \text{ grains}$$

Method (b)

$$\frac{35}{8} \times \frac{\text{total volume of diluted solution}}{\text{solution in fl oz}} \times \frac{100}{500} = \text{grains}$$

The total volume of diluted solution which can be made can be found by inspection. Since  $\frac{1}{8}$  fl. oz of concentrated solution makes 80 fl. oz of diluted solution, therefore, 8 fl. oz must make  $16 \times 80$  fl. oz.

The calculation, therefore becomes—

$$\frac{35 \times 16 \times 80 \times 100}{8 \times 500} = 1,120 \text{ grains}$$

#### EXERCISE 3.4

Calculate the number of grains required to make—

- 1 8 oz so that 2 teaspoonsfuls diluted to a quart will make a 1 in 5 000 solution
- 2 8 oz so that 3 teaspoonsfuls diluted to a pint make a 1 in 250 solution
- 3 10 oz so that 2 tablespoonsfuls diluted to 2 gallons will produce a 0.02 per cent solution
- 4 2 oz so that 10 minims in a wineglassful of water make a 1 in 200 solution (Capacity of wineglass is 2 oz)
- 5 16 oz so that 1 tablespoonful to half a gallon will make a 1 in 2,000 solution
- 6 8 oz so that 3 teaspoonsfuls to a pint will make a 0.0625 per cent solution
- 7 4 oz so that 1 teaspoonful to a quart makes a 0.0025 per cent solution
- 8 12 oz so that 3 teaspoonsfuls diluted to a pint will make an  $\frac{1}{8}$  per cent solution
- 9 6 oz so that 2 teaspoonsfuls to a pint will make a 1 in 2,500 solution
- 10 12 oz so that 1 tablespoonful diluted to half a pint will make a 0.04 per cent solution

Concentrated percentage solutions may be prescribed with directions to label appropriately for the

preparation of weaker percentage solutions (See Examples 3.19, 3.20 and 3.21.)

#### EXAMPLE 3.19

Send 8 oz of a 10 per cent solution of potassium permanganate, and label with directions for preparing a pint of a 1 in 400 solution

*Calculation—*

The first part of the calculation for preparing the solution is the same as in the previous examples

Thus—

35 gr, in 8 oz, will make a 1 per cent solution.

$35 \times 10$  gr, in 8 oz, will make a 10 per cent solution

$\frac{35 \times 10 \times 8}{8}$  gr, in 8 oz, will make a 10 per cent solution = 350 gr

Therefore 350 gr would be dissolved in water and diluted to 8 oz.

To find out how much of this will be required to make a pint of a 1 in 400 solution, the procedure is as follows—

A 10 per cent solution is 1 in  $\frac{100}{10} = 1$  in 10 solution

That is, 10 oz of the solution contains 1 oz of substance

10 oz of this solution diluted to 400 oz will produce a 1 in 400 solution

$\frac{10 \times 20}{400}$  oz of this solution diluted to 1 pint will produce a 1 in 400 solution =  $\frac{1}{2}$  oz

Therefore label the solution—

The Solution One tablespoonful to be diluted to a pint

*Check.* Note that 10 per cent is 40 in 400 and is, therefore 40 times stronger than the diluted solution which is 1 in 400. The 10 per cent solution must be diluted in this proportion, viz. one tablespoonful to one pint

---

## EXAMPLE 3 20

Send 20 oz of a 2.5 per cent solution, and label with directions for preparing a quart of 0.0625 per cent solution

*Calculation—*

35 gr, in 8 oz, will make a 1 per cent solution

$35 \times 2.5$  gr, in 8 oz, will make a 2.5 per cent solution

$\frac{35 \times 2.5 \times 20}{8}$  gr, in 20 oz, will make a 2.5 per cent solution

$$= 218.75 \text{ gr}$$

Therefore, 218.75 gr would be dissolved in water and diluted to 20 oz

A 0.0625 per cent solution is 1 in  $\frac{100}{0.0625} = 1$  in 1,600

A 2.5 per cent solution is 1 in  $\frac{100}{2.5} = 1$  in 40

That is, 40 oz of this solution will contain 1 oz of substance,

40 oz of this solution diluted to 1,600 oz will produce a 1 in 1,600 solution,

$\frac{40}{1,600}$  oz of this solution diluted to 1 oz will produce a 1 in 1,600 solution,

$\frac{40 \times 40}{1,600}$  oz of this solution diluted to 40 oz will produce a 1 in 1,600 solution = 1 oz.

Therefore label—

The Solution Two tablespoonsfuls to be diluted to a quart

*Check.* Note that 2.5 per cent is 40 times stronger than 0.0625 per cent. Therefore, the solution must be diluted in this proportion, viz. two tablespoonsfuls to one quart

---

## EXAMPLE 3 21

Occasionally, in the examination room, the order is written as follows—From the 8 per cent solution of mercuric chloride supplied, prepare 1 pint of a 1 in 1,000 solution

(continued overleaf)

*Example 3 21 continued**Calculation—*

This is, of course, exactly like the second part of the previous calculations

$$\text{An 8 per cent solution is 1 in } \frac{100}{8} = 1 \text{ in 12.5}$$

That is, 12.5 oz of this solution will contain 1 oz of mercuric chloride  
• 12.5 oz of this solution diluted to 1,000 oz will produce a 1 in 1,000 solution,

$$\cdot \frac{12.5}{1,000} \text{ oz of this solution diluted to 1 oz will produce a 1 in 1,000 solution,}$$

$$\frac{12.5 \times 20}{1,000} \text{ oz of this solution diluted to 20 oz will produce 20 oz of a 1 in 1,000 solution} = \frac{1}{4} \text{ oz}$$

Therefore, 120 minims of the 8 per cent solution would be diluted to 1 pint.

*Check.* Note that 8 per cent is 80 in 1,000 and must be diluted 80 times to produce a 1 in 1,000 solution, viz two teaspoonsfuls ( $\frac{1}{2}$  fl oz) to one pint

**EXERCISE 3 5**

Calculate the number of grains required to make—

- 1 8 oz of a 4 per cent solution and label with directions for preparing a quart of a 1 in 2,000 solution
- 2 3 oz of a 5 per cent solution and label with directions for preparing a pint of a 1 in 800 solution
- 3 16 oz of a 20 per cent solution and label with directions for preparing 2 gallons of a 1 in 1,600 solution.
- 4 20 oz of a 6 per cent solution and label with directions for preparing 30 oz of a 0.05 per cent solution
- 5 24 oz of a 16 per cent solution and label with directions for preparing 1 gallon of a 0.4 per cent solution
- 6 12 oz of a 20 per cent solution and label with directions for preparing half a gallon of a 1 in 400 solution.
- 7 How much of a 20 per cent solution will be required to make a quart of a 0.0625 per cent solution?
- 8 How much of a 15 per cent solution will be required to make 3 pints of a 0.4 per cent solution?

**WEIGHT IN WEIGHT PERCENTAGE SOLUTIONS**

As already mentioned on p. 14, percentage solutions of solids in liquids are not made w/w unless specially requested. The reasons for this are—

- (i) Solutions are almost invariably administered by volume, and it is therefore convenient, for comparison of dosage and other purposes, to have the quantity of solute directly and simply related to the volume and strength of the solution

- (ii) Equal volumes of *all* w/v solutions of the same strength contain the *same* amount of solute
- (iii) Equal volumes of w/w solutions of the same strength do not contain the same quantity of solute, owing to differences in the specific gravities of the liquids constituting the solvent

Taking a rather extreme example in order to illustrate this point, suppose that 100 ml each of a 5 per cent solution of iodoform in ether (sp gr 0.730), and a 5 per cent solution of camphor in chloroform (sp gr 1.490) were required.

The sp gr of a 5% w/w solution of iodoform in ether (0.730) is 0.777

Therefore, 100 ml of this solution weighs 77.7 G, and will contain 5% of 77.7 G = 3.885 G of iodoform in the 100 ml.

The sp gr of a 5% w/w solution of camphor in chloroform is 1.465

Therefore, 100 ml of this solution weighs 146.5 g and will contain 5% of 146.5 g = 7.325 g of camphor in the 100 ml.

If, then, there were no rule on the matter, these solutions might be prepared in two ways—

*Solution made w/w*    *Solution made w/v*

with sufficient solvent in each case to produce 100 ml.

with sufficient solvent in each case to produce 100 ml. It is reasonable to suppose that a prescriber would expect equal volumes of the above solutions to contain equal weights of the ingredients. Hence, to secure uniformity, all percentage solutions of solids in liquids are made w/v, this is the official method of calculation.

True percentage solutions (i.e. w/w) of solids in liquids are therefore sent only when specified.

### The general formula for weight in weight solutions

15—

The 'parts' of solute and solvent are both measures of mass, and all that is necessary is to use the

same denomination for both solute and solvent (i.e. G for both, grains for both, etc.)

The solution may be prepared either by adding the calculated weight of solute to the calculated weight of solvent, or by diluting the calculated weight of solute to weight with solvent, as convenient.

A little reasoning on the lines indicated in Example 3.22 will enable the student to find in any given case the approximate amount of solution to be made by weight, in order to fill a prescription where the quantity is ordered by volume.

A method sometimes given for calculating the quantities required for a w/w solution is to follow the method of calculation given for w/v solutions and then multiply the number of grains (or grammes) so found by the specific gravity of the solvent. This method is only approximately accurate for weak solutions say, up to 2 per cent, and is quite inaccurate for stronger solutions.

To illustrate if Example 3.22 were calculated in this way, the quantities would be—

2.5 G are required to make 50 ml of 5% w/v  
The specific gravity of the ether to be used is  
0.730

Therefore  $2.5 \text{ G} \times 0.730 = 1.825 \text{ G}$ , and this quantity would be dissolved in sufficient ether to produce 50 ml.

Now an accurate 5% w/w solution of iodoform in ether (0.730) prepared as stated under Example 3.24, has a sp gr of 0.777, so that 50 ml of it will weigh 38.85 G and will contain 5 per cent of 38.85 G of iodoform = 1.9425 G

There is therefore, a deficiency of

**EXAMPLE 3.22**

Send 50 ml of a solution containing 5% w/w of iodoform in ether

### **Calculation—**

A little consideration will show that as ether (methylated) has a sp. gr. of 0.730 a quantity considerably less than 50 G will measure 50 ml. Therefore conveniently prepare 40 G.

Weigh out 2 G of iodoform and add it to 38 G of ether weighed out in a stoppered bottle and shake until dissolved. From this quantity dispense the required 50 ml in a stoppered bottle.

If the order were for 2 oz of a 5% w/w solution of iodoform in ether, a similar plan would be followed—

If the solution had a sp gr about 1.00 then about  $437.5 \times 2$  gr = 875 gr would be needed to fill the prescription. But the sp gr of the solution will be low. Therefore, conveniently prepare 700 grains of solution.

Weigh out 35 gr of iodoform add to 665 gr of ether, weighed out in a stoppered bottle and shake until dissolved. From this quantity dispense the 2 fluid ounces required in a stoppered bottle.

$$(19425 - 1825) G = 01175 G,$$

equal to over 6 per cent deficiency

It is obvious that a volume of a correct w/w solution can be directly made only when the specific gravity of the finished solution, and not the solvent, is known.

As would be expected, admixtures or solutions of solids in solids are always made w/w. Admixtures or solutions of liquids in solids are also usually made w/w, this plan being more rational than v/w. Such preparations are exemplified by ointments, as in Example 3.23

### VOLUME IN WEIGHT PERCENTAGES

In spite of the fact that percentages of liquids in solids are usually made weight in weight in dispensing it should be noted that there is an official volume in weight percentage

*Per cent v/w (percentage volume in weight) expresses the number of millilitres of active substance in 100 grammes of product.*

The above is an official definition and, therefore, if a percentage in a prescription is qualified by the letters v/w it should be made according to the definition.

From the above it is clear that preparations made to the following formulae are identical in strength.

Liquid	1 ml
Solid to produce 100 G	
Liquid	1 fl oz
Solid to produce 100 oz	
Liquid	48 minims
Solid to produce 1 oz.	

The above are all 1 per cent v/w mixtures. It will be seen that since 48 minims in 1 oz (437.5 gr) is 1 per cent v/w, therefore, in 100 grains there will be

$$\frac{48 \times 100}{437.5} = 11 \text{ minims}$$

(See Example 3.25)

### EXAMPLE 3.23

#### Recipe—

Hydrg Ammon	2%
Liq Pic Carb	5%
Paraff Moll Flav ad	30 grammes
Fiat unguentum Signa—Nocte applicandum	

The quantities required will be—

Ammoniated mercury	0.6 G
Solution of Coal Tar	1.5 G
Soft Yellow Paraffin	27.9 G

### EXAMPLE 3.24

#### Recipe—

Acidi Salicylici	2
Amyli Pulverisatu	24
Zinci Oxidi	24
Paraffini Mollis	50

Mitte 3j Signa Pasta.

In order to obtain the quantities in whole numbers of grains for convenience in weighing it is preferable to make 500 gr, although only 480 gr are required to fill the prescription.

The quantities required, therefore will be—

Salicylic Acid	10 grains
Powdered Starch	"
Zinc Oxide	"
Soft Paraffin	250 ,

## EXAMPLE 3 25

Send 1 oz of Zinc Ointment containing 5% v/w of Coal Tar Solution  
 1 oz (avordupois) or 437 5 gr are required  
 $4\frac{8}{12}$  minimis in 1 oz = 1 per cent  
 $5 \times 4\frac{8}{12}$  minimis = 24 minimis are required

This quantity would be mixed with some of the Zinc Ointment and the product weighed. The remainder of the Zinc Ointment to produce 1 oz would then be weighed and incorporated

## Percentage Solutions of Liquids in Liquids

## VOLUME IN VOLUME SOLUTIONS

Percentage solutions of liquids in liquids are made v/v unless otherwise requested  
 The general formula for these is—

Liquid Solute      1 part by volume } for  
 Solvent, to produce 100 parts by volume } 1% v/v

The 'parts' of solute and solvent are both meas-

ures of capacity, and all that is necessary is to use the same denomination for both solute and solvent, i.e. ml for both, minimis for both, fluid oz for both, etc

In the metric system the general formula becomes

Liquid Solute      1 ml } for 1% v/v  
 Solvent, to produce      100 ml }

## EXAMPLE 3 26

Prepare 300 ml of a 5 per cent solution of Chloroform in Alcohol (90%)

*Calculation—*

$$\begin{array}{l} 1 \text{ ml with solvent to produce 100 ml is 1\% v/v} \\ \frac{1 \times 5 \times 300}{100} \quad " \quad , \quad 300 \text{ ml is 5\% v/v} \\ \qquad \qquad \qquad = 15 \text{ ml} \end{array}$$

15 ml of chloroform is therefore dissolved in sufficient alcohol (90%) to produce 300 ml

In the Imperial system the general formula may be expressed variously—

(a)	(b)	(c)
Liquid Solute	1 minim	4 8 minimis
Solvent to produce	100 minimis	1 fluid oz
		100 fluid oz
		(i.e. 480 minimis)
		v/v

(a) or (b) is a suitable basis for calculation when the quantity of solution required is small (c) is suitable for larger quantities

## EXAMPLE 3 27

Prepare 4 oz of a 2 per cent solution of Eucalyptus Oil in Olive Oil

*Calculation—*

$$\begin{array}{l} 4 \text{ fluid oz with solvent to produce 1 fluid oz is 1\% v/v} \\ 4 \times 2 \times 4 \text{ minimis} \quad " \quad " \quad 4 \text{ fluid oz is 2\% v/v} \\ \qquad \qquad \qquad = 38 \frac{1}{2} \text{ minimis} \end{array}$$

38½ or 39 minimis of eucalyptus oil is therefore dissolved in sufficient olive oil to produce 4 fluid oz.

**EXAMPLE 3 28**

Prepare 1 pint of a 25 per cent solution of Glycerin.

*Calculation—*

$$\begin{array}{rcl} 1 \text{ fluid oz with solvent to produce } 100 \text{ fluid oz is } 1\% \text{ v/v} \\ \frac{1 \times 20 \times 25}{100} & " & " \\ = 5 \text{ fluid oz. } 5 \text{ fluid oz of glycerin is therefore diluted to 1 pint.} \end{array}$$

**Alcohol Dilutions**

Alcohol (90%), or rectified spirit, contains 90 parts by volume of ethyl alcohol and 10 parts by volume of water and is, therefore, a true (v/v) percentage solution.

On mixing alcohol with water, contraction of volume and rise of temperature occur, and it is important, therefore, when preparing dilutions of alcohol, to dilute to volume after the mixture has cooled to the ordinary temperature. If 500 ml of 90 per cent alcohol are mixed with 500 ml of water, there is an appreciable rise in temperature. It will be noticed that the mixture frequently has a turbid appearance. This is caused by the evolution of minute bubbles of air, owing to its being more soluble in alcohol than in water, so that, on the addition of the latter, the air is partly expelled from

solution. The product, after cooling, will measure approximately 984 ml, and it should then be diluted with water to a litre, to produce 45 per cent alcohol. Alcohol dilutions may be calculated as shown in Example 3 29, from which it may be understood that, to calculate the amount of stronger alcohol required to make a weaker alcohol, the procedure is as follows—

Multiply the number of ounces (or ml) of diluted alcohol required by the percentage strength required, and divide by the percentage strength of the alcohol used.

Or, written as a formula—

$$\frac{\text{Volume required} \times \text{percentage required}}{\text{Percentage used}}$$

= { Volume of stronger  
alcohol to be used.

**EXAMPLE 3 29**

Prepare 600 millilitres of 60 per cent alcohol from 90 per cent alcohol provided.

Let  $n$  be the number of ml of 90 per cent alcohol required. These  $n$  ml will contain the same amount of alcohol as 600 ml of 60 per cent alcohol.

$$\text{i.e. } \frac{90}{100} \times n = \frac{60}{100} \times 600 = \frac{60 \times 600}{90} = 400 \text{ ml.}$$

**EXAMPLE 3 30**

Required to produce a pint of 60 per cent alcohol from 90 per cent alcohol.

Substituting figures in the above formula—

$$\frac{20 \times 60}{90} = 13\frac{1}{3} \text{ oz.}$$

Therefore dilute 13 oz 160 minimis with water to 20 oz at 15.5°C.

**EXERCISE 3 6**

Calculate the quantity of 90 per cent alcohol required to make—

- |                         |                          |
|-------------------------|--------------------------|
| 1 1 pint of 40% alcohol | 5 1 quart of 45% alcohol |
| 2 600 ml of 70% alcohol | 6 400 ml of 60% alcohol  |
| 3 8 oz of 60% alcohol   | 7 6 oz of 70% alcohol    |
| 4 500 ml of 20% alcohol | 8 24 oz of 20% alcohol   |

Calculate the quantity of 60 per cent alcohol required to make—

- |                           |                            |
|---------------------------|----------------------------|
| 9 1 gallon of 20% alcohol | 10 2 litres of 45% alcohol |
|---------------------------|----------------------------|

## COMBINED WEIGHT IN VOLUME AND VOLUME IN VOLUME CALCULATIONS

It is evident that formulæ identical with those obtained by transposition (e.g. those of Examples 3.2 and 3.3 and Exercise 3.1) will be obtained by applying the rules given above for the calculation of w/v and v/v percentage solutions.

It must be remembered that, unless otherwise

stated, solutions of solids in liquids are always made w/v, and solutions of liquids in liquids v/v.

It has already been shown that Method 1 (1 gr in 110 minimis is 1% w/v) for the calculation of w/v solutions is convenient when small volumes of weak solutions are required, because the quantity of solid needed is directly weighable. Method 1 may also be used when the solution contains liquid solutes stated as a percentage, it is, however, important to remember that 1 minim in 100 minimis (not 110 minimis) forms a 1% v/v solution.

### EXERCISE 3.7

1 Using the method of calculation described above, state the formula for—

- (a) 1 quart of Ammoniated Liniment of Camphor
- (b)  $\frac{1}{2}$  pint of Concentrated Peppermint Water

2 State the formula for the following—

*Recipe*—

Sodii Bicarbonatis	2
Boracis	2
Glycerini	10
Aquam	ad 100

Fiat collunarium Mitte 3 viii

3 State the formula for 300 ml of nosewash to the above formula

4 Using the method of calculation described above, state a suitable formula for the following—

*Recipe*—

Mentholis	2
Camphoræ	2
Thymolis	1
Olei Eucalypti	5
Paraffinum Liquidum Leve	ad 100

Fiat nebula Mitte 3 i

## MISCELLANEOUS PERCENTAGE CALCULATIONS

Orders for dilutions of acids and other compounds occasionally occur. In certain cases they may be of two types, and great care must be exercised to notice which is required.

For example, a request may be made for—

(a) 4 oz of an 8.25 per cent solution of acetic acid,

or (b) 4 oz of an 8.25 per cent solution of *real* acetic acid

Considering (a), it is apparent that, as acetic acid is the official name for an acid of a given strength, this request must be interpreted to mean a solution containing 8.25 per cent of this official

acid, and as this is a liquid, an 8.25% v/v solution of this would be supplied.

The calculation is—

8.25 volumes of acetic acid diluted to 100 volumes  
= 8.25% v/v of acetic acid

$\frac{8.25 \times 4}{100}$  fluid oz of acetic acid diluted to 4 fluid oz  
= 8.25% v/v of acetic acid

$\frac{33}{100}$  fluid oz = 158.5 minimis

Hence, 158.5 minimis would be diluted to 4 fluid oz.

Considering (b), as Acetic Acid B.P. contains 33% w/w of real acetic acid, it is sound to assume that an

8 25 per cent solution of real acetic acid of the same character, i.e w/w, is intended. As 8 25 per cent is a quarter of the strength of 33 per cent, the stronger acid will require to be diluted in the proportion of 1 part to 3 parts of water.

In order to obtain a w/w solution, these quantities must both be by weight, to measure them is inaccurate, as they have not the same specific gravity. To fill the above order, 1 oz by weight of acetic acid B.P. must be diluted to 4 oz by weight with water and this product sent. As 1 fluid oz of water weighs 1 oz avoirdupois, an alternative method is to weigh 1 oz avoirdupois of acetic acid and add 3 fluid oz of water.

The calculation of other strengths is similar to that for alcohol dilutions. That is—

Multiply the number of ounces (or grammes) of diluted acid required by the percentage strength required and divide by the percentage strength of the acid used.

Or, written as a formula—

$$\frac{\text{Weight required} \times \text{percentage required}}{\text{Percentage strength of acid used}}$$

$$= \left\{ \begin{array}{l} \text{Weight of stronger} \\ \text{acid to be used} \end{array} \right.$$

### EXAMPLE 3 31

Required, 8 oz of a solution of acetic acid containing 4 per cent of real acetic acid.

$$\text{Substituting figures in the above formula, } \frac{8 \times 4}{33} = \frac{32}{33} \text{ oz.}$$

It is unimportant whether troy or avoirdupois ounces are used provided that the same are used throughout. In the above, if troy ounces were available, then

$$\begin{aligned} \frac{32}{33} \text{ oz} &= \frac{32 \times 480}{33} \text{ grains} \\ &= 465.5 \text{ grains} \end{aligned}$$

Therefore, 465.5 gr of 33 per cent acid must be diluted to 8 oz troy, by weight.

If avoirdupois ounces only are available, then

$$\begin{aligned} \frac{32}{33} \text{ oz} &= \frac{32 \times 437.5}{33} \text{ grains} \\ &= 424.5 \text{ grains} \end{aligned}$$

Therefore, 424.5 gr of 33 per cent acid must be diluted to 8 oz avoirdupois, by weight.

### EXAMPLE 3 32

Prepare about 200 ml of a solution of ammonia containing 4 per cent by weight of ammonia.

The strong solution of ammonia of the B.P. contains 32.5 per cent of  $\text{NH}_3$  w/w.

Therefore,  $\frac{200 \times 4}{32.5}$  G of 32.5 per cent solution diluted to 200 G will

produce a 4 per cent solution of ammonia = 24.615 G.

Therefore 24.615 G of strong solution of ammonia must be diluted to 200 G and this product sent.

If the above request had read—

Prepare about 200 ml of a solution containing 4 per cent of Solution of Ammonia, the calculation would have been—

4 ml of Solution of Ammonia diluted to 100 ml = 4 per cent of the official solution.

8 ml of Solution of Ammonia diluted to 200 ml = 4 per cent of the official solution,

and this quantity would be prepared and sent

A similar kind of interpretation occurs with reference to solutions containing formaldehyde, and orders may be of two kinds

For example—

(a) Send 8 oz of a solution containing 4 per cent of Solution of Formaldehyde

This means a solution containing 4% v/v of the official solution

The calculation is—

4 volumes of official solution diluted to 100 volumes = 4% v/v of official solution,

$\frac{4 \times 8}{100}$  fluid oz of official solution diluted to 8 fluid oz = 4% v/v of official solution

$$= \frac{32}{100} \text{ fluid oz} = 153.6 \text{ minims}$$

Therefore, 153.6 minims would be diluted to 8 fluid oz

(b) Send 8 oz of a 4 per cent solution of formaldehyde

Liquor Formaldehydi B P contains 37 to 41 G of formaldehyde in 100 ml (i.e., w/v), and the 4 per cent solution required is therefore understood to be of the same character, that is, 4 G in 100 ml. The dilution must be made by diluting 4 volumes of the official solution to 39 volumes (the mean of 37 and 41). The formula used, therefore, is that given for alcohol dilutions

$\frac{8 \times 4}{39}$  fluid oz is the volume of the official solution required to make 8 fluid oz

$$= \frac{32}{39} \text{ fluid oz} = \frac{32 \times 480}{39} \text{ minims}$$

$$= 393 \text{ minims}$$

Therefore, dilute 393 minims to 8 fluid oz

### EXERCISE 38

Answers to calculations in the Imperial system, where the quantities are to be weighed, are to be given in terms of avoirdupois ounces

Calculate the amount of acetic acid B P required to make—

- 1 20 oz of solution containing 5 per cent of real acetic acid
- 2 4 oz of solution containing 20 per cent of real acetic acid
- 3 250 ml of solution containing 15 per cent of real acetic acid
- 4 Calculate the amount of strong solution of ammonia required to make 8 oz of solution containing 2 per cent of ammonia
- 5 Calculate the amount of lactic acid of the pharmacopœia required to make 2 oz of solution containing 10 per cent of lactic acid
- 6 Calculate the amount of official solution of formaldehyde required to make half a gallon of a solution containing 2 per cent of formaldehyde (The official solution to be taken as containing 39% w/v of formaldehyde)

**EXERCISE 3.9****For Revision—**

- ✓ 1 Calculate the number of grains required to make 3 oz of solution of zinc sulphate so that 1 teaspoonful diluted to a pint will make a 1 in 250 solution
- 2 Calculate the number of grains required to make 4 oz of a solution of thymol in alcohol (90%), so that 1 teaspoonful to half a tumblerful of water will make a 1 in 2,000 solution (Capacity of tumbler = 10 oz)
- 3 Calculate the number of grains required to make 2 oz of a 2 per cent solution of boric acid in glycerin
- 4 Calculate the number of grains required to make 12 oz of a solution of alum so that 2 teaspoonfuls to a quart will make a  $\frac{1}{2}$  per cent solution
- 5 Calculate the number of grains required to make 2 oz of a 10 per cent solution of thymol in rectified spirit
- 6 Calculate the number of grains required to make 20 oz of a stock solution of mercuric chloride so that 1 oz diluted to a pint will make a 1 in 1,000 solution
- 7 Calculate the number of grains required to make 1 oz of 20 per cent solution of salicylic acid in collodion
- (8) Calculate the amount of lactic acid of the pharmacopoeia required to make 3 oz of a solution containing 15 per cent of lactic acid
- 9 State the number of grains required to dispense—

*Recipe—*

Argenti Nitratis	5%
Aquam Purif . . . . .	ad 3 j

- 10 20 oz of a solution containing 2 per cent of tannic acid and 0.1 per cent of mercuric chloride is required. Calculate the weight of each that must be used
- 11 Calculate—1 oz of a 5 per cent solution of resorcin.
- 12 What will be the weight of alum and zinc sulphate required to make 100 tablets so that 1 tablet dissolved in a pint of water will make a solution containing 0.25 per cent of alum and 0.2 per cent of zinc sulphate?
- 13 Calculate—1 pint of a 1 in 150 solution of sodium bicarbonate
- 14 Calculate the number of grains required to make 6 oz of a solution of potassium permanganate so that 2 teaspoonfuls to a pint will make a 1 in 4,000 solution
- 15 How much of a 5 per cent solution of mercuric potassium iodide will be required to make half a gallon of 1 in 500?
- 16 How much acetic acid B.P. is required to make 4 oz of a solution containing 15 per cent of real acetic acid?
- 17 Calculate—10 oz of a 0.2 per cent solution of silver nitrate
- 18 Calculate the number of grains required to make 8 oz of a 2 per cent solution of borax
- 19 Calculate the number of grains required to make 10 oz of a solution of zinc sulphate so that 1 tablespoonful diluted to 2 pints will make a 1 in 160 solution
- 20 What will be the weight of sodium chloride required in each powder so that 1 powder to a pint of water will make normal saline solution (0.9%)
- 21 Calculate the amount of creosote required to make 4 oz of a 2.5 per cent solution of creosote in rectified spirit

- 22 Calculate the number of grains required to make 8 oz of a solution of copper sulphate so that 1 tablespoonful diluted to a half pint of water will produce a  $\frac{1}{2}$  per cent solution.
- 23 Calculate the amount of chloroform required to make 3 oz of a 10 per cent solution of chloroform in alcohol.
- 24 How much 90 per cent alcohol will be required to make 700 ml of 20 per cent alcohol?
- 25 How much 90 per cent alcohol will be required to make 12 oz of 60 per cent alcohol?
- 26 Give a suitable formula for 1 oz of a 0.125 per cent solution of Zinc Chloride in Adrenaline Solution and Distilled Water, equal parts
- 27 Find, by transposition, and the use of a suitable multiple or submultiple, the formula for—  
 1 pint of Compound Glycerin of Thymol B.P.C.  
 1 pint of Mixture of Ammonia and Ipecacuanha B.P.C.
- 28 Find by percentage calculation, the formula for—  
 1 pint of Syrup of Codeine Phosphate B.P.C.
- 29 Give a suitable formula for 4 drachms of a  $\frac{1}{2}$  per cent solution of atropine sulphate

### PROPORTIONAL CALCULATIONS AND ALLIGATION

One example of this type of calculation involves the mixing of two similar preparations, but of different strengths, to produce a preparation of intermediate strength (See Example 3.33)

#### EXAMPLE 3.33

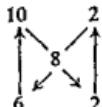
100 grammes of a powder containing 8 per cent of substance  $x$  are to be made from powder containing 10 per cent of  $x$  and powder containing 2 per cent of  $x$ .

Let  $n$  be the number of grammes of the 10 per cent powder required. Then  $100 - n$  grammes will be the weight of 2 per cent powder required. Therefore,

$$\begin{aligned} \frac{10}{100} \times n + \frac{2}{100} \times (100 - n) &= \frac{8}{100} \times 100 \\ 10n + 200 - 2n &= 800 \\ 8n &= 600 \\ n &= 75 \end{aligned}$$

i.e. 75 grammes of the 10 per cent powder will be mixed with 25 grammes of the 2 per cent powder

This type of calculation may be done by a method known as *alligation*. The quantities are set out as follows and Example 3.33 is here used as an illustration



Subtract 8 from 10 = 2 (required percentage from stronger percentage)

Subtract 2 from 8 = 6 (weaker percentage from required percentage)

Then 6 parts of the 10 per cent powder plus 2 parts of the 2 per cent powder will produce 8 parts of 8 per cent powder. 100 grammes are required

Therefore, quantity of 10 per cent powder required

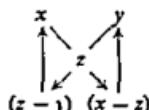
$$= \frac{100}{8} \times 6 = 75 \text{ G}$$

and quantity of 2 per cent powder required

$$= \frac{100}{8} \times 2 = 25 \text{ G}$$

Although it can be seen that this method produces the correct answer it is not recommended, except as a method of checking since its logic is not immediately obvious and if a slip in writing a figure is made it may not be readily apparent. An algebraical proof of the method is given below.

These methods may also be used when one component is an inert diluent such as lactose or water



i.e. we take  $(z-j)$  of  $x$  and  $(x-z)$  of  $y$  to give the required strength

Hence if true we have

$$(z-j)x + (x-z)y = (z-y+x-z)$$

$$\text{i.e. } zx - xj + xj - zy = -zy + xz$$

This is true therefore the method is correct

#### PROOF OF ALLIGATION METHOD

Let  $x$  and  $y$  be the percentage strength of constituents and let  $z$  be the percentage strength of the product required. Then, by alligation

#### EXAMPLE 3.34

Prepare 1 000 grammes of Dilute Acetic Acid from Acetic Acid.

Acet. c Acid B.P.  $\approx 33\%$  w/w

Dilute Acetic Acid B.P.  $= 6\%$  w/w

Let  $n$  be the number of grammes of 33 per cent acid required. Then

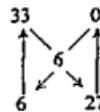
$$\frac{33}{100} \times n = \frac{6}{100} \times 1000$$

$$33n = 6000 \quad \text{and} \quad n = \frac{6000}{33} = 182 \text{ G}$$

It should be obvious that this may be written down in the first instance as—

$$\frac{6}{33} \times 1000 = 182 \text{ G}$$

Check by alligation—



i.e. subtract 0 (for water) from 6  $\Rightarrow 6$

Subtract 6 from 33  $\Rightarrow 27$

Then 6 parts by weight of Acetic Acid 33 per cent plus 27 parts by weight of water will give 33 parts by weight of Dilute Acetic Acid 6 per cent. 1 000 grammes are required.

$$\begin{aligned} \text{therefore the quantity of } 33\% \text{ acid required} &= \frac{1000}{33} \times 6 \\ &= 182 \text{ G} \end{aligned}$$

$$\text{and the quantity of water required} = \frac{1000}{33} \times 27 = 818 \text{ G}$$

## WEIGHING SMALL QUANTITIES

It is accepted dispensing practice never to attempt to weigh less than 1 grain or 50 mg, and such small quantities should be weighed on an accurate chemical balance. Quantities less than the above amounts may be obtained by the methods indicated below. Other examples will be found elsewhere in the book by reference to the Index under 'Small doses'.

### Apothecaries' System

#### *Fractional Quantities in Powders*

When a fractional quantity is required in a powder, a diluent such as lactose, will be required to prepare a dilution from which an aliquot portion may be weighed.

For example, to obtain  $\frac{1}{2}$  grain of strychnine hydrochloride, weigh 1 grain ( $\frac{5}{2}$ ) and dilute to 5 grains by adding 4 grains of lactose. Each grain of this mixture or triturate will contain  $\frac{1}{2}$  grain of strychnine hydrochloride,  $\frac{1}{2}$  grain will therefore, be contained in 2 grains. Such triturations should be made with great care as described on p 79.

For fractional quantities greater than 1 grain take the nearest weighable quantity greater than the amount required. For example, to obtain  $2\frac{1}{2}$  grains of morphine sulphate, weigh  $2\frac{1}{2}$  grains.  $2\frac{1}{2}$  grains contains  $20 \times \frac{1}{2}$  grain, therefore, dilute this to 20 grains with lactose. Each grain of this dilution contains  $\frac{1}{2}$  grain, therefore, to obtain  $2\frac{1}{2}$  ( $1\frac{1}{2}$ ) grains, 19 grains should be weighed.

#### *Fractional Quantities in Solution*

These may be obtained similarly by making suitable solutions and taking aliquot portions.

For example, to obtain  $3\frac{1}{2}$  grains of copper sulphate, weigh  $3\frac{1}{2}$  grains.  $3\frac{1}{2}$  grains contains  $14 \times \frac{1}{2}$  grain, therefore, dissolve this quantity in 14 volumes of water. Since 1 volume contains  $\frac{1}{2}$  grain, to

obtain  $3\frac{1}{2}$  ( $1\frac{1}{2}$ ) grains, 13 volumes should be measured.

The units of volume chosen, minims, drachms or fluid ounces, should be as large as possible to avoid the error involved in measuring small quantities. The solubility of the substance may also affect the volumes chosen.

### Metric System

#### *Fractional Quantities in Powders*

In the metric system quantities less than 0.05 grammes may easily be obtained by making a 1 in 10 or 1 in 100 dilution with lactose or other suitable diluent and taking the appropriate aliquot portion. For example, to obtain 0.035 grammes of atropine sulphate, weigh 0.05 grammes and dilute to 0.5 grammes by adding lactose. This is a 1 in 10 dilution, therefore, 0.035 grammes of the salt will be contained in  $10 \times 0.035$  grammes, i.e. 0.35 grammes of triturate.

#### *Fractional Quantities in Solution*

Usually a 1 in 1,000 solution is a suitable strength to prepare. This solution, containing 1 mg in 1 ml simplifies the calculation involved, and is made by dissolving 50 mg in 50 ml.

For example, to obtain 0.015 G of mercuric oxycyanide, dissolve 50 mg and adjust to 50 ml. 0.015 grammes (15 mg) will be contained in 15 ml of the solution.

Where the final volume of the required solution does not allow of so great a dilution, a 1 in 100 solution may be made. It is obvious that in the above example, if the final solution were only 10 ml a more concentrated solution would be required. A solution containing 0.05 G in 5 ml, i.e. 10 mg in 1 ml could be prepared and 1.5 ml of this would contain the necessary 15 mg.



# 4

## Mixtures

Medicines in liquid form constitute a large class of remedial agent for internal administration, and the following are some reasons for their popularity

- 1 They are more quickly effective than, for example, pills or tablets, which require previous disintegration in the body before absorption can begin
- 2 Certain substances can only be given in liquid form, the character of the remedy, or the large dose, makes administration in any other form inconvenient, e.g. castor oil, liquid paraffin.
- 3 The usefulness of some substances is largely dependent upon administration in a diffused form. For example, light Kaolin is used to adsorb toxic substances in the gut, the great surface area of the fine powder giving maximum adsorbent effect
- 4 Certain chemical substances, e.g. potassium iodide and bromide, may cause pain if taken in the dry state as a powder or tablet

The present chapter deals with medicines in liquid form, with the exception of (a) Emulsions, which are discussed in Chapter 5, and (b) Mixtures involving

Incompatibility, which are considered in Chapter 16, when the student's knowledge of the reactions of drugs and chemicals is more advanced.

### Definition

A Mixture is a liquid medicine for internal use, of which several doses are contained in one bottle (distinction from a 'draught')

### CLASSIFICATION

For the purpose of these exercises, mixtures are classified according to the following plan—

- Class 1 Simple Mixtures containing Soluble Substances only
- Class 2 Mixtures containing Diffusible Solids
- Class 3 Mixtures containing Indiffusible Solids
- Class 4 Mixtures containing Precipitate forming Liquids
- Class 5 Mixtures containing Slightly-soluble Liquids
- Class 6 Miscellaneous Mixtures.

### Class 1. Simple Mixtures containing Soluble Substances Only

#### METHOD FOR SIMPLE MIXTURES

##### STAGE 1

Dissolve the solids in about three-quarters of the vehicle (see below). Solution may be effected thus—

*Substances in List A* (p. 41) Simply add the material, without previous powdering, to the vehicle in a measure, and stir until dissolved.

*Substances in List B* (p. 42) Finely powder the material in a mortar, add the vehicle, and stir until dissolved. Then transfer the solution to a measure, rinse the mortar with a small quantity of vehicle, and add to the contents of the measure.

The term 'vehicle' is used to denote the liquid in which the medicinally-active ingredients are dis-

solved or suspended. The vehicle may be inert (e.g. water), or a flavouring agent (e.g. Peppermint Water), or a preserving and sweetening agent (e.g. Chloroform Water), or it may have some medicinal property (e.g. Infusion of Senna). The preparation of those vehicles which are made extemporaneously will be explained as they arise.

Three-quarters of the vehicle is selected as a suitable proportion for Stage 1, for the following considerations—

- (a) The volume occupied by the other ingredients will rarely exceed the remaining one-quarter, but, if it should, the quantity of vehicle used in Stage 1 must be reduced.

(b) Solution is hastened by using as much of the solvent as convenient

#### STAGE 2

Examine the solution *critically* by holding it against the light. If particles of foreign matter are visible, strain the solution through a plug of cotton wool placed in a funnel, and, when the solution has passed through, pour a *little* of the aqueous vehicle on the wool so that the solution therein is displaced

#### STAGE 3

Add any liquid ingredients. After measuring each, rinse the vessel used with a *little* of the aqueous vehicle, and add to the contents of the measure.

*Liquids in List C* (p. 42) These contain very volatile substances, consequently precautions must be taken to minimize loss by volatilisation

Thus, the vehicle used in Stage 1 may have been heated to facilitate solution of the solid. In such cases the liquid must be thoroughly cooled before the addition of any liquid containing a volatile substance. Further, the succeeding Stages 4 and 5 must be completed as quickly as possible, to minimise loss by volatilisation from the open measure. Where practicable it is preferable to adjust the mixture to volume *minus* the volume of the volatile liquid, transfer to the bottle, add the volatile liquid, and cork immediately.

#### STAGE 4

Add more of the vehicle to produce the prescribed volume

Adjustment to volume should be made in a graduated measure, because of the frequent discrepancy between the reputed and actual capacity of dispensing bottles.

The stirrer must, of course, be removed from the measure before adjusting to volume

#### STAGE 5

Transfer the mixture to the bottle, cork, and then thoroughly polish the bottle to remove finger-marks. Attach the label, wrap the bottle, and address it

#### Soluble Substances

A number of water-soluble substances in common use appears in Lists A and B (pp. 41 and 42). Some of these substances are much less soluble than others, but they are administered in smaller dose, or in weaker solutions, so that, as ordinarily prescribed, they will dissolve. For example, 1 part of Potassium Citrate dissolves in 1 part of water, the maximum

dose is 60 grains, hence this amount readily dissolves in sufficient aqueous vehicle to produce  $\frac{1}{2}$  oz—this being the usual volume of an adult dose. For Quinine Hydrochloride, 23 parts of water are needed to effect solution of 1 part of the compound. The maximum dose is, however, only 10 grains, hence this quantity will dissolve in the aqueous vehicle needed to form the  $\frac{1}{2}$ -oz dose.

Hence, although the substances in Lists A and B differ widely in comparative solubilities, they are all soluble as usually prescribed.

#### LIST A

*Substances in common use which do not require powdering before use. If occurring in a simple mixture, they readily dissolve with stirring.*

Ammonium Bicarbonate	Phenol
Ammonium Bromide	Potassium Acetate
Ammonium Chloride	Potassium Bicarbonate
Atropine Sulphate	Potassium Bromide
Caffeine and Sodium Iodide	Potassium Citrate
Calcium Chloride	Potassium Iodide
Chloral Hydrate	Potassium Permanganate
Citric Acid	Quinine Bisulphate
Cocaine Hydrochloride	Quinine Dihydrochloride
Dextrose Monohydrate	*Sodium Acid Phosphate
Ephedrine Hydrochloride	Sodium Benzoate
Hexamine	Sodium Bicarbonate
Homatropine Hydrobromide	Sodium Bromide
Hyoscine Hydrobromide	Sodium Carbonate
Hyoscyamine Hydrobromide	Sodium Chloride
Iron and Ammonium Citrate	Sodium Citrate
Lithium Citrate	Sodium Iodide
*Magnesium Sulphate	Sodium Metabisulphite
Morphine Hydrochloride	Sodium Nitrite
Morphine Tartrate	Sodium Potassium Tartrate
Pancreatin	Sodium Salicylate
Pepsin	*Sodium Sulphate
*Phenazone	Strontium Bromide
Phenobarbitone	Strychnine Hydrochloride
Sodium	Tannic Acid
	Tartaric Acid
	Urea
	Zinc Sulphate

\* Dissolve more readily in warm solvent

## LIST B

*Substances in common use which do not readily dissolve unless finely powdered in a mortar before the aqueous vehicle is added*

Alum	Ferrous Sulphate
Amidopyrin	Mercuric Chloride
*Borax	*Potassium Chlorate
*Boric Acid	*Potassium Nitrate
Caffeine Citrate	Quinine Hydrochloride
*Calcium Lactate	Sodium Phosphate

## LIST C

*Liquids which are either very volatile, or which contain volatile substances or gases in solution*

Tincture of Chloroform and Morphine B P C	Aromatic Spirit of Ammonia B P C.
Ether	Compound Spirit of Orange B P C.
Ethereal Tincture of Lobelia B P C.	

## Containers for Mixtures

Plain bottles should be used for preference. Moulded graduations on large bottles may be sufficiently accurate for ordinary mixtures, but where the capacity is small, and the fine divisions numerous, accurate dosage cannot be obtained from the grad-

uations—these being moulded equidistant, regardless of the varying internal diameter of the bottle.

Similarly, the measurement of doses in the domestic teaspoon or tablespoon may admit variation of nearly 100 per cent (see p. 9) and it is therefore desirable for the patient to use a graduated medicine glass. Although the words 'teaspoonful' and 'tablespoonful' are used on such measures, they are based upon one-eighth and one half of an ounce respectively.

In all children's mixtures, in all cases where an unusual dose is given, and, thirdly, when the full dose of a potent remedy is prescribed, it is necessary to call attention to the dose by underlining it and by prefixing the word 'measured,' i.e. a measured teaspoonful.

It occasionally happens that the prescribed dose of a mixture is not expressed in teaspoonsfuls or table spoonfuls and in such cases it is necessary to place a narrow strip of gummed paper at the back of the bottle, and to mark thereon the level of each dose. The label should indicate the dose, e.g. one-sixth part, etc.

The bottle selected should then be fitted with a cork. Until visual proficiency is attained, this fitting should be done before the medicine is put into the bottle so that unsuitable corks may be returned to stock for later use. The ideal fit should leave about two-thirds of the cork projecting from the neck of the bottle, to ensure easy removal.

## EXERCISE 4 1

## Recipe—

Potassii Bromidi	3 n
Tinctura Nucis Vomicae	3 n
Aquam Chloroformi	ad 3 vi

\* Fiat mustura. Signetur Cochlearie magnum ter in die post cibos sumendum

Follow the *Method for Simple Mixtures* on pp. 40 and 41. Notice that Potassium Bromide is in List A.

Observe the order of work mentioned under General Dispensing Procedure p. 4

Assistance in translating the directions to the prescriptions in this and the succeeding chapter is provided in Appendix 6.

## EXERCISE 4 2

## Recipe—

Ammonii Bicarbonatus	gr v
Tinctura Ipecacuanhae	m x
Extractus Scenega Liquidi	m v
Aquam Menthae Piperitae	ad 3 ss

Fiat mustura. Sigma Ter in die capienda. Mitte 3 iv

Observe the previous order of work, and follow the *Method for Simple Mixtures*, pp 40 and 41 Add the Peppermint Water ( $\frac{1}{4}$  of the vehicle i.e. 3 oz), as directed for Stage 1, and complete the *Method for Simple Mixtures* (p 41)

*Note on Vehicle for Exercise 4.2*

Official Aromatic Waters e.g. Peppermint Water, Cinnamon Water, may be made in two different ways—

1 *By solution*

2 *By diluting the concentrated water* This is 40 times the strength of ordinary aromatic water, indicated on the label, thus Conc 1-39, meaning 1 volume added to 39 volumes of distilled water will produce the ordinary strength Hence  $\frac{480}{40} = 12$  minims of concentrated aromatic water must be diluted to 1 oz by the addition of distilled water to produce the ordinary strength aromatic water

Should Peppermint Water not be available, prepare 4 oz from Concentrated Peppermint Water for this exercise, and reject the excess

### EXERCISE 4.3

*Recipe—*

Sodii Bicarbonatis	3 <i>i</i>
Tinctura Nucis Vomicae	m <i>lxxx</i>
Infusum Gentianae Compositum	ad <i>3 iv</i>

Fiat mustura Signa Cochleare amplum bis in die ante cibos capendum

Observe the previous order of work, and follow the *Method for Simple Mixtures* (p 40) for dispensing this prescription Note that Sodium Bicarbonate is in List A

*Note on Vehicle for Exercise 4.3*

Concentrated Infusions are used in such cases These are eight times stronger than ordinary infusions the label usually reads Conc 1-7, meaning that 1 volume added to 7 volumes of Purified Water produces the ordinary strength of infusion

Use Concentrated Compound Infusion of Gentian for this prescription, dilute 4 drachms of it to 4 oz with Purified Water to produce ordinary strength infusion use from this the amount required for the prescription, and reject the unused portion

### Class 2 Mixtures containing Diffusible Solids

Diffusible solids are those which do not dissolve in water, but may be mixed therewith so that, upon shaking, the powder is evenly diffused throughout the liquid for sufficient time to ensure uniform distribution in each dose]

They may be—

- (a) Insoluble (List D), or
- (b) Slightly Soluble (List E)

For simplicity in classification, certain soluble substances appear in List E as slightly soluble, because, in the proportion usually prescribed, they may not completely dissolve in the vehicle This partial solubility is for convenience, ignored, and the

substances are classified as diffusible solids although in certain proportions they may entirely dissolve

Most substances dissolve more freely in hot water than in cold, but when the water is cooled to room temperature it may throw out some of the previously dissolved substance, in a crystalline form This must be carefully guarded against, because of the impossibility of evenly re-dissolving the substance when measuring the dose, and also because crystals are unpleasant in the mouth Consequently, the vehicle must be used cold and any part of the substance which does not completely dissolve will be present as a fine diffusible powder

**METHOD FOR DIFFUSIBLE SOLIDS****STAGE 1**

*Finely powder the substance (if not already in fine powder), using a mortar, add any soluble substances, and mix. Measure about three-quarters of the vehicle, pour a portion of it into the mortar containing the powder, and triturate to form a smooth cream, then gradually add the remainder of the vehicle measured out.*

Some of the substances in List D are already in fine powder and will diffuse evenly by stirring, but the uniform method of trituration in a mortar should be generally adopted.

The presence of air in the interstices of many powders, particularly those of vegetable origin, causes some of the powder to float on the water. To prevent this tendency, make a smooth cream first, by adding only a small quantity of vehicle, and then diluting.

**STAGE 2**

Examine the contents of the mortar critically, and if particles of foreign matter are visible, spread a piece of muslin over the mouth of a measure, and pour the contents of the mortar through it. Rinse the mortar with a little of the vehicle, and then pass the liquid through the muslin. Alternatively, if only one or two foreign particles are visible, remove them with a glass rod and omit straining through muslin.

**STAGE 3**

As Stage 3 of the *Method for Simple Mixtures*, p. 41

**STAGE 4**

As Stage 4 of the *Method for Simple Mixtures*, p. 41

**STAGE 5**

As Stage 5 of the *Method for Simple Mixtures*, p. 41

**Particular Substances****LIST D**

*Substances and preparations which are diffusible*

Aromatic Chalk Powder	
Bismuth Carbonate	
Bismuth Subnitrate	
Compound Bismuth Powder	
Compound Kaolin Powder	
Compound Liquorice Powder	
Compound Magnesium Trisilicate Powder	
Compound Rhubarb Powder	
*Light Kaolin	
*Magnesium Carbonate, Light or Heavy	
*Magnesium Oxide, Light or Heavy	
Magnesium Trisilicate	
Phenolphthalein	
Quinine Sulphate	
Rhubarb, powdered	

**LIST E**

*Substances commonly prescribed in a quantity greater than will dissolve*

	1 part soluble in	Equal to a man- num of (approx.)
†Borax	†20 parts of water	†20 grains per oz
†Tartaric Acid	†20 "	†20 "
Caffeine		
Citrate	32 "	13 "
Calcium		
Lactate	20 "	20 "
Potassium		
Chlorate	14 "	30 "

Solubility may be increased by the presence of added substances—for example, those marked with an asterisk are freely soluble in glycerin, so that, when this is present, complete solution may be effected.

\* The light variety is used where not otherwise stated. When Kaolin is prescribed without qualification, Light Kaolin must be supplied.

† These figures need not be memorised provided the aqueous vehicle is used at room temperature if the amount of solid dissolves, a simple mixture ensues; if the solid does not dissolve entirely the undissolved amount is diffusible hence the *Method for Diffusible Solids* can be applied uniformly without actual knowledge of the solubility of the substance

**EXERCISE 4****Recipe—**

Magnesu Sulphatus	3 1
Magnesu Carbonatus	3 1
Aquam Menthae Piperite	ad 3 vi
Fiat mustura Signa Cochlearia ampla duo semihora ante jentaculum sumenda	

Follow the *Method for Mixtures containing Diffusible Solids* (p 44)

The Magnesium Sulphate and Carbonate will together displace about  $\frac{1}{2}$  oz of liquid (i.e. about half their own weight), therefore prepare  $5\frac{1}{2}$  oz of Peppermint Water from the concentrated water (p 43), and reject the excess

### EXERCISE 4.5

#### Recipe—

Acidi Borici	1 G
Tinctura Hyoscyami	2 ml
Infusum Auranti	ad 15 ml

Fiat mixtura Signa Bis in die sumenda Mitte doses sex

As shown in List E, 1 G of Boric Acid requires 25 ml of water for complete solution, hence only a part of the quantity ordered on the above prescription will dissolve Follow the *Method for Mixtures containing Diffusible Solids* (p 44)

Prepare 80 ml Infusion Orange Peel by diluting 10 ml of the official concentrated infusion with Purified Water

### Class 3. Mixtures containing Indiffusible Solids

A solid is regarded as indiffusible when it will not remain evenly distributed in the vehicle long enough to ensure uniformity of the measured dose. The vehicle must, therefore, be increased in viscosity, and it should be noticed that the viscosity required to hold a given powder in suspension for a stated period is almost independent of the quantity of the powder. Therefore the amount of suspending agent required depends upon the volume of the mixture.

The two best suspending agents for general use are—

(a) Compound Tragacanth Powder

The proportion to be used is—

Imperial System      Metric System

10 grains per oz of mixture    2 G per 100 ml of mixture

(b) Tragacanth Mucilage

The proportion to be used is—

One-quarter of the volume of the mixture

The former must always be used when the vehicle is other than water or Chloroform Water (because Tragacanth Mucilage is prepared with Chloroform Water), to avoid replacement of some of the medicinally active vehicle by the mucilage.

### METHOD FOR INDIFFUSIBLE SOLIDS, USING COMPOUND TRAGACANTH POWDER

#### STAGE 1

Finely powder the indiffusible substance in a mortar, add any soluble or diffusible solids and the Compound Tragacanth Powder and mix intimately

Measure about three-quarters of the vehicle, triturate the powders with a portion of it until a smooth cream is formed, and then add the remainder of the vehicle measured out

#### STAGE 2

Examine the contents of the mortar critically. If any particles of foreign matter are visible, spread a piece of muslin over the mouth of a measure, and pour the contents of the mortar through it. Rinse the mortar with a little of the vehicle, and then pass the liquid through the muslin. Alternatively, if only one or two foreign particles are visible, remove them with a glass rod and omit straining through muslin

#### STAGE 3

As Stage 3 of the *Method for Simple Mixtures*, p 41

#### STAGE 4

As Stage 4 of the *Method for Simple Mixtures*, p 41

#### STAGE 5

As Stage 5 of the *Method for Simple Mixtures*, p 41

### METHOD FOR INDIFFUSIBLE SOLIDS, USING TRAGACANTH MUCILAGE

#### STAGE 1

Finely powder the indiffusible substance in a mortar, add any soluble or diffusible solids, and mix intimately

Triturate the material with the Tragacanth Mucilage to form a smooth cream, and then gradually dilute with half the vehicle. The product will thus measure about three-quarters of the finished volume, as in previous instances.

Complete as described above from Stage 2.

#### Particular Substances

##### LIST F

*Substances which require suspending when ordered in mixture form*

*Acetylsalicylic Acid	Podophyllum Resin
Barbitone	Prepared Chalk
Benzoic Acid	Quinidine Sulphate
Bismuth Oxychloride	Quinine Salicylate
Bismuth Salicylate	Salicylic Acid
Calomel	Salol
Chlorbutol	Scammony Resin
Jalap Resin	Succinylsulphathiazole
Phenacetin	Sulphadimidine
Phenobarbitone	

\* Dissolves in presence of alkali citrates.

#### EXERCISE 4 6

##### Recipe—

Cretæ Preparatæ	gr xxx
Tincture Catechu	m xx
Aquæ Cinnamomi	ad 3 i

Fiat mustura Signa Secundis horis sumenda Mitte doses sex  
Prepared Chalk is in List F, and a suspending agent is therefore indicated. Follow the Method for Indiffusible Solids, using Compound Tragacanth Powder (p 45).

#### EXERCISE 4 7

##### Recipe—

Phenacetini	3 G
Caffeinae	1 G
Syrupi Aurantii	12 ml
Aquam	ad 90 ml

Fiat mustura Signa Cochlearie magnum ex lacte dolore urgente sumendum.

Phenacetin is in List F, and a suspending agent is therefore necessary. The vehicle is water, and either Compound Tragacanth Powder or Tragacanth Mucilage may, therefore be used. For practice, follow the Method for Indiffusible Solids, using Tragacanth Mucilage (p 45).

#### Class 4 Mixtures containing Precipitate-forming Liquids

Examination of List F will show several resins. They are not only insoluble in water, but often form indiffusible masses, particularly when salts are present (see p 51).

Certain liquid preparations, enumerated in List G, contain resinous matter, and, when mixed with water, the resin is precipitated and may adhere to the sides of the bottle, or form a clotted precipitate which will not re-dissolve upon shaking. To prevent this it is necessary in mixtures of this type to add Compound Tragacanth Powder or Tragacanth Mucilage in the same proportion as for indiffusible powders (p 45).

It must always be used when the vehicle is medicinally active.

##### STAGE 1

Finely powder in the mortar any indiffusible solid, add those diffusible, and then the Compound Tragacanth Powder. Mix thoroughly. If no solid ingredients are required in the prescription, place the Compound Tragacanth Powder in the mortar alone.

Measure three-quarters of the vehicle, and triturate the powders with sufficient to form a smooth cream; gradually add the remainder of the vehicle measured out.

Measure the precipitate-forming liquid in a dry measure, and pour in a slow stream into the centre of the cream, stirring rapidly all the time.

Then add any soluble ingredients and stir until dissolved.

#### METHOD FOR PRECIPITATE-FORMING LIQUIDS, USING COMPOUND TRAGACANTH POWDER

This method is very convenient where diffusible or indiffusible solids are also present in the mixture, and

The above procedure is adopted because it is important to precipitate the particles of resin in a colloidal state, and to stabilise and protect them with the protective colloid, Compound Tragacanth Powder, before adding the soluble solids, which are in many cases, electrolytes (*vide p 51*)

#### STAGE 2

Examine the contents of the mortar critically. If any particles of foreign matter are visible, spread a piece of muslin over the mouth of a measure, and pour the contents of the mortar through it. Rinse the mortar with a little of the vehicle, and then pass the liquid through the muslin. Alternatively, if only one or two foreign particles are visible, remove them with a glass rod and omit straining through muslin.

#### STAGE 3

As Stage 3 of the *Method for Simple Mixtures*, p 41

#### STAGE 4

As Stage 4 of the *Method for Simple Mixtures*, p 41

#### STAGE 5

As Stage 5 of the *Method for Simple Mixtures*, p 41

#### METHOD FOR PRECIPITATE-FORMING LIQUIDS, USING MUCILAGE OF TRAGACANTH

This method is more rapid than the other, and may be used when insoluble solids are absent, and the vehicle is water, or chloroform water (because Tragacanth Mucilage is made with chloroform water)

#### EXERCISE 4 8

#### Recipe

Potassii Iodidi	ʒ ss
Tincturae Lobeliae Aetheriae	ʒ ii
Tincturae Stramonii	ʒ iv
Aquam Chloroformi	ad ʒ vi

Fiat mixtura Signa Cochlearie amplum quater in die sumendum

Aetherial Tincture of Lobelia is in List G, and a suspending agent is, therefore, indicated. The vehicle is water and there are no insoluble solids, therefore follow the *Method for Precipitate-forming Liquids, using Tragacanth Mucilage* p 45

#### Class 5. Mixtures containing Slightly-soluble Liquids

It has already been noticed on p 43 that certain slightly soluble solids are often prescribed in excess of their solubility, and that the undissolved portion

#### STAGE 1

Mix the Tragacanth Mucilage with an equal volume of the aqueous vehicle. Measure the precipitate-forming liquid in a dry measure, and pour slowly into the centre of the mixture, with constant stirring.

As mentioned above, it is important to precipitate the resin and stabilise the colloidal particles of it with Tragacanth Mucilage before soluble solids are added.

#### STAGE 2

Dissolve any solids in one quarter of the aqueous vehicle. Strain if necessary, and add to the above mixture, which will now form about three-quarters of the final volume, as in previous instances.

#### STAGES 3, 4, AND 5

As for Simple Mixtures, p 41

#### Particular Substances

##### LIST G

*Resinous tinctures and other preparations which yield an undissolved precipitate with an aqueous vehicle, and therefore require a suspending agent—*

Ammoniated Solution of Quinine B P C—the precipitate in this instance is quinine (hydrated)	Tincture of Benzoin B P C.
Compound Benzoin Tincture	Tincture of Lobelia, Ethereal B P C
	Tincture of Myrrh B P C

Tincture of Tolu B P C

Note Tincture of Myrrh does not need a suspending agent (except when salts are present in appreciable proportion), because the precipitated resin remains in a diffusible form.

is diffusible provided it is in the form of a fine powder

The insoluble portion of slightly soluble liquids,

on the other hand, is not readily dissoluble, and a suspending agent is therefore necessary. Compound Tragacanth Powder and Tragacanth Mucilage are used for this purpose, in the proportions directed for indissoluble solids (p. 45).

### METHOD FOR MIXTURES CONTAINING SLIGHTLY-SOLUBLE LIQUIDS, USING TRAGACANTH MUCILAGE

This method is convenient when insoluble solids are absent, and the vehicle is not medicinally active.

#### STAGE 1

Place the slightly-soluble liquid in a bottle, add the mucilage and shake vigorously.

#### STAGE 2

Dissolve any soluble ingredients in half the aqueous vehicle, filter if necessary and add it gradually to the contents of the bottle, shaking thoroughly after each addition. If soluble solids are absent, use half the aqueous vehicle in the same way.

Transfer the liquid from the bottle to a measure-

#### STAGE 3

As Stage 3 of the *Method for Simple Mixtures*, p 41

#### STAGE 4

As Stage 4 of the *Method for Simple Mixtures*, p 41

#### STAGE 5

As Stage 5 of the *Method for Simple Mixtures*, p 41

### METHOD FOR MIXTURES CONTAINING SLIGHTLY-SOLUBLE LIQUIDS, USING COMPOUND TRAGACANTH POWDER

This method is necessary when insoluble solids are present, or when the vehicle is medicinally active.

#### STAGE 1

Finely powder the insoluble solids in a mortar, add any soluble solids and the Compounds Tragacanth Powder and mix intimately. If there are no solids, place the Compound Tragacanth Powder in a mor-

tar. Measure out three-quarters of the aqueous vehicle, add sufficient to form a smooth cream, then add the slightly-soluble liquid, and mix thoroughly. Gradually add the remainder of the measured out quantity of aqueous vehicle.

Critically examine the liquid, if necessary strain through muslin into a measure, and rinse the mortar with a small quantity of the aqueous liquid, and add to the contents of the measure.

#### STAGE 2

As Stage 2 of the *Method for Simple Mixtures*, p 41

#### STAGE 4

As Stage 4 of the *Method for Simple Mixtures*, p 41

#### STAGE 5

As Stage 5 of the *Method for Simple Mixtures*, p 41

#### Particular Substances

##### LIST H

*Slightly-soluble Liquids which are likely to be prescribed in excess of solubility—*

		Equal to an approximate maximum of
Creosote	150 parts of water	3 minutes per oz
Paraldehyde	9 " "	48 "

Note. The above are very soluble in alcohol, and Creosote is very soluble in glycerin, hence the prescribed quantity may entirely dissolve if in favourable proportion. In such cases the mixture becomes a *Simple Mixture*, the liquid being dissolved in the solvent, and the solution added at Stage 3.

#### EXERCISE 4.9

##### Recipe—

Paraldehyde	3 <i>fl</i>
Syrup	3 <i>fl</i>
Extractus Glycyrrhizae Liquidus	3 <i>ss</i>
Aquam	ad 3 <i>fl</i>

Fiat haustus Mitte duo Signa Unus more dicto sumendus

Reference to List H will show that the Paraldehyde is present here in slight excess of solubility.

The vehicle is inert, and there are no insoluble solids, therefore follow the *Method for Mixtures containing Slightly-soluble Liquids, using Tragacanth Mucilage* above.

### Class 6. Miscellaneous Mixtures

#### 1. Mixtures containing Small Doses of Potent Medicaments

The single doses of many substances is a small fraction of a grain, and the total quantity prescribed may not be weighable directly, as, for instance—

*Recipe—*

Hyoscine Hydrobromide . . . . gr  $\frac{1}{160}$   
Aquam Chloroformi ad  $\frac{1}{5}$  fl

Fiat mustura Sigma Ter die sumenda Mitte  $\frac{1}{3}$  m

There are 12 doses of gr  $\frac{1}{160}$ , giving a total of  $\frac{1}{5}$  of a grain of Hyoscine Hydrobromide. Weights of this denomination are not made, and the procedure in these circumstances is as follows—

Dissolve the smallest weighable quantity greater than that required (i.e. the nearest  $\frac{1}{2}$  grain above—*minimum 1 grain*) in a volume of the aqueous vehicle which will ensure that the portion of the solution to be used can be measured simply and accurately.

On the above prescription, the smallest weighable quantity above  $\frac{1}{5}$  of a grain is 1 grain. When this 1 grain is dissolved,  $\frac{1}{5}$  of the solution formed will contain the requisite  $\frac{1}{5}$  grain of the drug whatever volume of solvent is used. In choosing the volume to be used, it is most important, owing to the potency of the drug, to fix a quantity which will be directly measurable both in itself, and also in the actual portion to be used. Neither 2 minims nor 25 minims is a graduation line on minim measures, and accurate\* measurement cannot easily be obtained (unless, of course, a minim pipette is available). Con-

sequently a much larger volume of vehicle (Chloroform Water) must be used as a solvent for the potent drug (Hyoscine Hydrobromide). Suitable volumes would be—

(a) 250 minims of solvent, using 20 minims of the solution

250 minims = 240 + 10, 240 is a line graduation on a 1-oz measure, and 10 on a minim measure  
20 minims is marked on the latter

(b) 25 drachms, using 2 drachms of the solution

#### 2. Use of Official Solutions to obtain Small Doses

The Pharmacopoeia and the British Pharmaceutical Codex include a few solutions of potent substances, e.g.—

Arsenical Solution containing 1.0% w/v of arsenic trioxide

Adrenaline Solution, containing 0.1% w/v of adrenaline

Strychnine Hydrochloride Solution containing 1.0% w/v of strychnine hydrochloride

Morphine Hydrochloride Solution containing 1.0% w/v of morphine hydrochloride

These are convenient in suitable cases where fractions of a grain are required (Examples 4 1, 4 2)

#### 3. Calcium Lactate Mixtures

The solubility of Calcium Lactate is officially given as 1 in 20. Although the composition of different samples of this substance, is fairly uniform, the solubility varies from 1 in 12.9 to 1 in 26.5, according to the method of manufacture. Hence, about 30 grains of one sample may dissolve in an ounce, and

\* An error of only  $\frac{1}{2}$  minim in measuring 2 minims is more than 12 per cent in error.

#### EXAMPLE 4 1

The total amount of Morphine Hydrochloride required in a prescription may be  $\frac{1}{16}$  grain. The official solution which is available contains 1% w/v, i.e. 1 grain in 110 minims; therefore  $\frac{1}{16}$  grain will require 154 minims. This volume may be measured in different ways, e.g. 150 + 4—the 150 by direct measurement, and the 4 by diluting a further 10 minims to 100 minims, and measuring 40 minims of this dilution.

#### EXAMPLE 4 2

The total quantity of Adrenaline required in a prescription may be  $\frac{1}{16}$  grain.

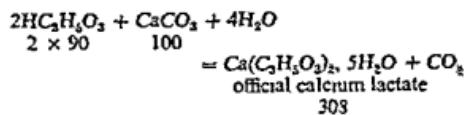
The official solution contains 0.1% w/v, i.e. 1 grain in 1,100 minims.

For  $\frac{1}{16}$  grain, measure  $\frac{5 \times 1,100}{16}$  i.e. 344 minims, along the lines directed above.

only 16 grains of another. This causes a variation in the appearance of a mixture, sometimes it may be clear, and sometimes there may be a diffused powder. Some commercial specimens have the objectionable odour of sour milk to an undesirable extent.

When freshly made, this odour is not so apparent, and solubility is also good—consequently it may be prescribed in this form (*Calcii Lactas Recens*). Calcium carbonate and lactic acid are allowed to interact, to form this product. Lactic Acid B P contains lactic acid and lactide, together equalling not less than 87.5 per cent w/w of real lactic acid.

If the official acid is simply diluted with water and excess of calcium carbonate added, only the real lactic acid present will react to form calcium lactate. If, therefore, the excess of calcium carbonate were filtered off, the filtrate would contain calcium lactate and facetae. Upon storage, part of the facetae would undergo hydrolysis to form lactic acid, and the mixture would acquire a sour taste. Hence, apart from the fact that the relative proportions of real lactic acid and lactide vary, and consequently prevent exact calculation, it is important to select a method of preparation which will ensure almost complete conversion of the lactide to calcium lactate. It has been shown that this occurs when the two are boiled together for about 20 minutes. The reaction, and the quantities, can be found from the equation—



The weight per ml of the official acid is 1.20, and contains the equivalent of 87.5 per cent w/w of real acid, therefore the quantity of official acid needed for 100 G of Calcium Lactate B P will be—

$$\frac{180 \times 100 \times 100}{308 \times 87.5} = 66.8 \text{ G} \quad \text{or} \quad \frac{66.8}{1.20} = 55.2 \text{ ml.}$$

Similarly, 67 grains, or  $\frac{67 \times 110}{100 \times 1.20} = 61 \text{ minims}$ , will be required for 100 grains of Calcium Lactate B P.

The theoretical amount of calcium carbonate needed for 100 G of Calcium Lactate B P will be—

$$\frac{100 \times 100}{308} = 32.4 \text{ G, or } 32\frac{1}{2} \text{ grains for 100 grains}$$

An appreciable excess of calcium carbonate should, however, be present, to ensure conversion of

the lactide to calcium lactate, and this will be provided by using 40 G (or 40 gr)

#### Recipe—

Calci Lactatus Recensis	3 ii
Syrupi Aurantii	3 vi
Aquam Cinnamomi	ad 3 vi

Fiat mustura Signa Cochlearia magna duo bis  
in da sumenda

#### Calculation

From the above considerations it will be seen that the required quantities are as follows—

Precipitated Calcium Carbonate	48 gr
Lactic Acid B P	73 minims

#### METHOD

Dilute the acid with about 4 oz of distilled water (three-quarters of total volume), add the calcium carbonate, and boil for about 20 minutes. Cool slightly, filter to remove excess of calcium carbonate, and wash the filter with a small quantity of water. The combined volume of Calcium Lactate and Orange Syrup may be taken as 1 oz, i.e. the mixture contains 5 oz of Cinnamon Water. Therefore add 60 minims of Concentrated Cinnamon Water and the Orange Syrup to the filtrate, and then adjust to volume with distilled water.

#### 4 Other Forms of Mixtures

##### DROPS

These may be remedies given in their original form without dilution, perhaps because of decomposition in aqueous media. Hence such liquids are properly prescribed either alone, or with alcoholic liquids, Compound Cardamom Tincture being often used for this purpose.

Special attention must be given to the labelling of these preparations, as there is a considerable difference between 'drops' and 'minims'. The following table shows the number of drops in 60 minims of certain liquids—

Alcohol	170
Belladonna Tincture	150
Chloroform	292
Distilled Water	70
Nux Vomica Tincture	163
Opium Tincture	152
Spirit of Camphor	169

Thick viscous liquids produce large drops, because the drops adhere to the surface of the glass until the

weight overcomes the power of adhesion. Consequently the greater the surface area of the dropper, the larger the drop. Hence a standard dropper should be used, having an external diameter of 3 mm (8 in approx.)

Drops are sent out in plain bottles or vials, accompanied by a standard dropper or a measure graduated in minims.

#### DRAUGHTS

These do not differ from mixtures, except that usually there are only one to three doses, and they are sent out in separate bottles.

#### Protective Effect of Gums

In some suspensions, e.g. salol in water, most of the individual particles are large enough to be seen by the naked eye; in others, e.g. bismuth carbonate in water, they are visible only in mass, and upon standing they deposit. When acacia, for instance, is added to water, it appears to be in solution but it has been shown that there are minute suspended particles too small to be seen even under a microscope, and it does not, therefore, form a true solution like salts. Matter in this microscopic state of division is said to be in a colloidal state.\*

Colloidal particles have many special properties and it is of interest in this section to note their behaviour with electrolytes, e.g. magnesium sulphate, potassium bromide, and salts generally.

The addition of an electrolyte to certain colloidal solutions causes rapid agglomeration of the particles, with formation of large visible particles—the colloid is then said to be precipitated. With other colloidal solutions, e.g. a solution of acacia or tragacanth no such precipitation takes place, as will be noticed in the following experiments—

*Expt 1* Dilute 10 ml of Acacia Mucilage with 40 ml of water, and add 10 ml of a 10 per cent solution of sodium chloride—no precipitation takes place.

*Expt 2* Add 5 ml of Tincture Myrrh to 45 ml of water, forming an opalescent liquid containing colloidal particles of the resin of myrrh. Now add 10 ml of a 10 per cent solution of sodium chloride as before—within a few minutes a curdy precipitate is formed, which ultimately rises to the surface. The precipitate consists of agglomerated resin.

A solution of acacia is a protective colloid, i.e. not only is it stable with electrolytes, but it also

#### LINCTUSES

These are preparations usually containing medicaments having a local action on the mucous membrane of the throat. The vehicle is some mucilaginous, syrupy, or viscous substance, and, to ensure prolonged action, they are sipped or swallowed slowly without dilution. Being simple solutions or admixtures, their preparation presents no difficulty.

#### ELIXIRS

These are sweet aromatic preparations often containing a proportion of alcohol—they are mostly simple solutions or admixtures.

#### on Colloidal Precipitates

prevents precipitation of other colloids when an electrolyte is added thereto. This may be demonstrated in the following manner—

*Expt 3* Add 5 ml of Tincture of Myrrh and 10 ml of Acacia Mucilage to 35 ml of water. Now add 10 ml of a 10 per cent solution of sodium chloride—there is no precipitation, although without the acacia (*Expt 2*) a precipitate is formed.

In simple language, the protective effect may be explained by saying that the colloidal particles of acacia surround the particles of the other colloid, forming an effective barrier against the electrolyte. It is evident that the acacia or other protective colloid must be present when the electrolyte is added—no protective action can ensue if the electrolyte has already agglomerated the colloid. To illustrate—

*Expt 4* Add 10 ml of Acacia Mucilage to the product formed in *Expt 2*. There will be no change in the character of the precipitate, although it will not so readily separate at the surface, because of the increased viscosity of the mixture.

Hence the reason for the procedure on pp. 46–47—the resinous tincture must be precipitated in the presence of Tragacanth Mucilage or Compound Tragacanth Powder, before the electrolyte is added.

The value of different protective colloids varies widely. Gelatin possesses high protective properties, but its tendency to putrefactive decomposition renders it unsuitable for general use. Acacia is a better protective than tragacanth, but the latter affords adequate protection for the colloidal particles formed from resinous tinctures. Compound Tragacanth Powder contains both acacia and tragacanth, and also starch, which itself has protective properties.

\* An account of colloidal solution is given in *Tutorial Pharmacy*.

## STOCK SOLUTIONS

It is quicker to measure a liquid than to weigh a solid, hence *stable* soluble salts in frequent use are often made into solutions of known strength.

A common strength for very soluble substances is 1 in 3, meaning that 1 grain is contained in 3 minims of solution, or 1 drachm in 3 fluid drachms of solution. Less soluble substances are necessarily stocked in weaker solutions, e.g. 1 in 6, 1 in 8.

The use of stock solution, correctly prepared and filtered, replaces Stages 1 and 2 in dispensing 'Simple Mixtures,' and shortens the work in a busy pharmacy. It must be remembered, however, that measuring is less accurate than weighing and great care must be taken to ensure a satisfactory degree of accuracy.

### PREPARATION

It is important to notice that 3 fluid ounces (1,440 minims) of a 1 in 3 solution must contain 480 grains of the salt. If apothecary's ounces are available, 1 apothecary ounce of salt is dissolved in sufficient water to produce 3 fluid ounces of solution. If only avordupois ounces are available, the necessary correction must be made. The avordupois ounce contains 437.5 grains, hence 42.5 grains must be added to each, to represent one apothecary ounce.

Preparation is usually effected by simple stirring, or by using a warm solvent. After adjustment to volume, the solution should be strained through a

plug of cotton wool, or if necessary filtered through filter paper.

### ERRORS INVOLVED IN THE USE OF STOCK SOLUTIONS

Capper and Smith (1960) carried out a series of experiments in the use of stock solutions in dispensing. Their conclusions included the claim that, using standard procedures, these solutions can be made to within a tolerance of ( $\pm$ ) 2 per cent if full allowance is made for the B.P. and B.P.C. tolerances for the drugs.

They point out, however, that another and greater source of error is involved in measuring volumes of stock solutions in dispensing individual mixtures. This involves a further variation of  $\pm 5$  per cent.

The authors conclude that, if the use of stock solutions is to be regarded as good pharmaceutical practice, a reasonable tolerance for solids so dispensed would be ( $\pm$ ) 7.5 per cent, which is greater than the tolerances allowed for B.P.C. mixtures which is usually ( $\pm$ ) 5 per cent.

The student is recommended to refer to experiments, recorded by the following, all of which are concerned with errors involved in measuring liquids: Capper, Cowell and Thomas (1955), Parkinson and Rogers (1956), Capper and Dare (1957) and Farrell, Smith and Fitchett (1961).

## STOCK MIXTURES

These are mixtures, prepared in bulk, to enable smaller volumes to be dispensed quickly when required. The use of these is generally frowned upon as being unethical and liable to lead to inaccuracy. It is, however, a fact that stock mixtures are widely used and nothing is achieved by ignoring them.

Stock (1960) gives an analytical survey of 420 samples of stock mixtures used by 118 Birmingham pharmacists. As a result of his investigations he makes certain very positive and challenging suggestions, such as, the recognition and official approval of stock mixtures together with the preparation of a stock mixture formulary.

Logically, if mixtures so dispensed comply with the proper standards, no serious objection to their use can be made. There are, however, serious objections to most stock mixtures and these are outlined below.

Stock mixtures are either 'single strength' or 'concentrated' and may be solutions or suspensions

There is also a practice of using a *Pulv. pro Mist.*, i.e. mixed powders from which a mixture is prepared as required by weighing out an appropriate quantity and suspending it in the required volume of vehicle together with any liquid ingredient.

*Single-strength Stock Mixtures.* Provided these are solutions of substances which do not deteriorate in solution there can be no objection to their use. Large volumes can be made with greater accuracy than small volumes. Winchester bottles are sometimes used to prepare 80 fluid ounce quantities of these, and care must be taken in carefully calibrating the bottle.

*Stock Mixture Suspensions.* These introduce a source of error due to the possibility of inadequate shaking to distribute the suspended particles thoroughly before drawing off the required volume. It is not always easy to determine that a completely homogeneous suspension has been obtained.

*Concentrated Stock Mixtures.* The use of these is

open to criticism, even if they consist of stable solutions, since, added to the possible inaccuracy in their preparation are the added errors involved in measuring quantities of them and adjusting to volume in dispensing. Measuring, it has already been said, is less accurate than weighing.

Concentrated suspensions as stock mixtures are still further open to criticism since, added to the above errors are the errors of inadequate mixing before measuring an aliquot portion.

*'Pulv pro Mist'* The practice of using mixed powders from which quantities are weighed to produce a suspension is open to serious condemnation since there is a real danger of powders of different density separating during the handling of the container. Quantities weighed out may, therefore, be of varying composition.

To sum up it would appear that in satisfactory pharmaceutical practice stock mixtures are justified only in the case of single strength stable solutions.

The mixtures of the B.P.C. are intended for extemporaneous dispensing and for use when freshly prepared. They are not designed for concentration as stock mixtures. If the use of stock mixtures is to be accepted they should be properly formulated for

that purpose and accepted with stated tolerances and storage times.

#### REFERENCES

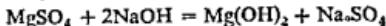
- BATESON, F R C (1931) Comparison of Certain Suspending Agents *Pharm J* 126, 52-53
- BRINDLE, H (1931) Calcii Lactas Recens *Pharm J* 127, 80-81
- CAPPER, K R, COWELL, D B and THOMAS, J A (1955) The Measurement of Liquids, *Pharm J* 175, 241-246
- CAPPER, K R and DARE, J G (1957) Dispensing Tolerances in Liquid Medicines *Pharm J* 179, 231-239
- FARRELL, F G, SMITH, G and FITCHETT, E J (1961) Stock Solutions of Potassium Citrate *Pharm J* 186, 45-46
- JACKSON, J (1934) The Dispensing of Mixtures Containing Volatile Ingredients *Pharm J* 132, 387-388
- MACMORRAN, G H (1933) The Solubility of Calcium Lactate *Pharm J* 130, 245
- PARKINSON, J C and ROGERS, A R (1956) Errors in the Measurement of Water *Pharm J* 176, 382
- SMITH, G (1960) Examination of a Number of Dispensing Balances *Pharm J* 185, 473-478

### OFFICIAL MIXTURE

#### Magnesium Hydroxide Mixture (*Syr* Cream of Magnesia)

This is a suspension of magnesium hydroxide in water. Part of the magnesium hydroxide which it contains is prepared by precipitation, the remainder by the hydration of light magnesium oxide. The method of preparation is—

A solution of sodium hydroxide is triturated with light magnesium oxide to form a suspension free from lumps. After dilution this cream is added to a solution of magnesium sulphate, which is stirred throughout the whole period of admixture. The following reaction takes place—



Further, the magnesium oxide becomes hydrated—



a process requiring upwards of 48 hours for completion.

The mixture is then set aside for the magnesium hydroxide to settle. After pouring off the clear layer above the precipitate the latter is washed with Purified Water until the washings yield only a slight

reaction for sulphate. Washing may be effected on a calico filter or by decantation. Sufficient Purified Water is then added to the magnesium hydroxide to form the prescribed volume of product.

The magnesium hydroxide is prepared in part by precipitation and in part by hydration, in order to produce a mixture—

- (i) which will not be unduly viscous, and
- (ii) in which the magnesium hydroxide will not readily subside—in the ideal cream of magnesia the magnesium hydroxide should not settle, leaving a clear liquid above even after standing for several days.

A cream of magnesia can be made by simply triturating light magnesium oxide with water to form a smooth cream, diluting to volume and setting aside for two days. This product, upon keeping usually becomes gelatinous and unpourable. A cream of magnesia containing magnesium hydroxide, prepared by precipitation only, separates somewhat rapidly, but remains fluid. A cream containing magnesium hydroxide, prepared by precipitation, and by hydration meets the above requirements.

REVISION EXERCISES 1 *Recipe*—

Ammonii carbonatus gr v  
 Tincturae Scillæ m x  
 Potassii Iodidi gr v  
 Infusum Senegæ Recens B P 1932 ad  $\frac{3}{1}$

Fiat mixtura Signa Quartis horis capienda Mitte  $\frac{5}{1}$  v.

2. *Recipe*—

Mixt Lobeli et Stramon. Co  
 Mitte  $\frac{5}{1}$  viii

Sig — ss p r n.

## 3 Prepare 2 oz of Compound Senna Mixture B P C

4. *Recipe*—

Mixt Kaolin pro Inf  
 Mitte  $\frac{5}{1}$  iii

Sig —  $\frac{5}{1}$  ii bis in die sumendum

5. *Recipe*—

Mixt Pot Cit et Hyoscyr  
 Mitte  $\frac{3}{1}$  viii

Sig —  $\frac{3}{1}$  ss t d.s

6. *Recipe*—

*Acidi Acetysalicylici* gr x  
*Potassii Citratis* gr xxx  
*Aquam Chloroformi* ad  $\frac{3}{1}$  i

Fiat mixtura Signa Ter in die sumenda. Mitte  $\frac{5}{1}$  vi

7. *Recipe*—

*Pulveris Cretæ Aromatici*  $\frac{3}{1}$   
*Spiritus Ammonii Aromatici*  $\frac{5}{1}$   
*Tincturae Catechu*  $\frac{3}{1}$  ii  
*Auam Cinnamomi* ad  $\frac{3}{1}$  iv

Fiat mixtura Signa Cochleare magnum quater in die capendum.

8. *Recipe*—

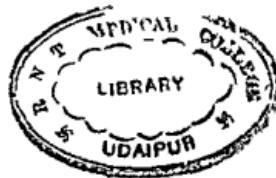
*Salolis* gr v  
*Bismuthi Salicylatus* gr xv  
*Aquam* ad  $\frac{3}{1}$  i

Fiat mixtura Signetur Bis in die sumenda Mitte  $\frac{5}{1}$  iv

9. *Recipe*—

Mixt. Rhei, Ammon. et Sod.  
 Mitte  $\frac{5}{1}$  viii

Sig —  $\frac{5}{1}$  ss nocte sumendum.



## Emulsions\*

WHEN two immiscible liquids are in contact with one another there exists at the separating surface, or interface, a certain tension or force which retards dispersion of one liquid in the other, and the liquids are accordingly said to be immiscible. Vigorous shaking may break up one liquid into globules, which become temporarily distributed throughout the other liquid. For example, castor oil and water are immiscible, but upon shaking together the oil is broken up into small globules, which become temporarily dispersed in the water. The oil is then in the *disperse* phase, and the water, which forms a continuous system around the oil, is in the *continuous* phase. This condition is only temporary, as separation quickly takes place upon standing.

If, however, a third substance is added, it may concentrate as a film at the interface. As a consequence, the globules of the dispersed liquid may remain indefinitely distributed in the other liquid, instead of coalescing to form one separate layer. A substance which can act in this manner is called an emulsifying agent, and the product is an emulsion, which may be briefly defined as a mixture containing two immiscible liquids, one of which is broken up into minute globules, each surrounded by a film of the emulsifying agent, and dispersed throughout the other liquid (Fig. 51, p. 67).

Some emulsions may contain the oil in the disperse phase, i.e. in the form of tiny globules dispersed throughout the water, and these are referred to as the *oil-in-water* type (see Fig. 51(a), p. 67).

Other emulsions may contain the water in the disperse phase, and the oil in the continuous phase, these are called *water-in-oil* emulsions (see Fig. 51(b), p. 67).

The oil-in-water type are produced by such emulsifying agents as gum acacia, methylcellulose, saponins, soaps formed from a monovalent base (K, Na, NH<sub>3</sub>), and numerous synthetic substances. For the water-in-oil type, wool fat, resins, beeswax, soaps derived from a divalent base (Ca, Mg, Zn) and synthetic compounds are commonly used. Both types are represented in pharmaceutical emulsions, of which those intended for internal use are preferably of the oil-in-water type—the reason being that, by surrounding the oil globules with water, their unpleasantness is considerably reduced.

### Emulsifying Agents

For extemporaneous production of an emulsion for internal use there is nothing to excel Acacia. For emulsions to be used externally the common emulsifying agents are the various emulsifying waxes, potash or lime soaps—soap not being suitable when acid is present, owing to decomposition and liberation of fatty acids.

The use of a single emulsifying agent is quite satisfactory for most dispensing work, but it has been proved that sometimes two, or perhaps three, agents together will produce the finest emulsion and this is usually the practice for large scale manufacture.

### CLASSIFICATION

The exercises included in this chapter are based upon the classification of emulsions according to the agent used—

#### Natural Gums

- Class 1 Acacia
- Class 2 Mucilage of Irish moss
- Class 3 Tragacanth

\* See also Ointment Emulsions on page 136 *et seq.*  
Theoretical aspects of emulsions and emulsifying agents are dealt with in *Tutorial Pharmacy*

**Gum Substitutes**

Class 4 Cellulose ethers and other cellulose derivatives

Class 5 Salts of alginic acid

**Soaps**

Class 6 Various soaps

**Saponins**

Class 7

**Starch**

Class 8

**Natural Waxes**

Class 9 Wool fat

Class 10 Beeswax

**Synthetic Waxes**

Class 11 Emulsifying Wax, Cetrimide Emulsifying Wax, Cetomacrogol Emulsifying Wax.

The above classification of emulsifying agents is

most satisfactory for this practical book, but modern classifications usually group them in a different manner. Subdivision is made into non ionic, anionic, and cationic groups, although a number of naturally occurring substances cannot be included in these groups. This classification, fully explained in *Tutorial Pharmacy*, is briefly outlined below.

*Non ionic* emulsifying agents are, of course, those which do not ionize, e.g. polyethylene glycol esters of fatty acids and polyethylene glycol ethers of fatty alcohols.

*Anionic* emulsifying agents are well exemplified by soaps such as sodium oleate, in which the anion contains the characteristic group (oleic) which confers emulsifying properties on the compound. Sulphated and sulphonated compounds also belong to this group, e.g. sodium lauryl sulphate.

*Cationic* emulsifying agents are represented by the quaternary ammonium compounds, such as cetyl trimethyl ammonium bromide, in which the characteristic group with emulsifying properties (cetyl) is in the cation.

The terms *anion active* and *cation active* are also applied to these two latter groups.

**Class 1 Acacia Emulsions**

Acacia in fine powder is a very satisfactory emulsifying agent for general use, and the proportions used are shown in Table 51.\*

**METHOD FOR SIMPLE EMULSIONS****STAGE 1**

Measure the oil in a dry measure, and thoroughly triturate with the powdered acacia in a dry mortar.

**STAGE 2**

Using a clean, dry measure, take exactly twice as much aqueous vehicle as there is gum, add it all at once to the contents of the mortar and rapidly

triturate without ceasing until a thick cream is obtained.

It is most important to continue trituration until the product is white, or nearly so. When the correct stage is reached there is usually a clicking sound upon brisk trituration. At this stage the product is called the primary emulsion.

**STAGE 3**

Calculate the approximate quantity of aqueous vehicle required to complete the volume, and add it gradually to the primary emulsion with constant trituration. Transfer the emulsion to a suitable measure and adjust exactly to volume, rinsing out the mortar with additional aqueous vehicle if required. Finally, stir to make the emulsion uniform.

\* This is called the 'Dry Gum Method'. See also the alternative 'Wet Gum Method' on p. 59.

Table 51

FOR FIXED OILS	FOR VOLATILE OILS	FOR OLEO-RESINOUS SUBSTANCES
<i>Use quarter as much gum as oil</i> The more common fixed oils prescribed in emulsions are Almond, Castor, Cod liver, and Olive Oils, and Liquid Paraffin.	<i>Use half as much gum as oil</i> Volatile oils which may be prescribed are Oil of Turpentine, Oil of Cubeb, Sandalwood Oil, and Terebene.	<i>Use an equal amount of gum</i> Copaiba Balsam of Peru, and Extract of Male Fern are typical of this class.

*Note* Acacia always contains dust. This may be removed by straining through muslin or by setting the emulsion aside for ten minutes and decanting.

### Containers for Emulsions

While emulsions for internal use are sent out like mixtures, it is desirable to use a wide mouthed bottle for thick liquids.

Emulsions may be labelled 'The Mixture' or 'The Emulsion,' as preferred, but in all cases a 'Shake the Bottle' label should be attached, because a concentrated emulsion may form at the surface (p. 71) and this must be redistributed by shaking before use.

For emulsions intended to be used externally, distinctive bottles, preferably bearing the words 'Not to be Taken moulded in the glass, must always be used. The label should bear the words 'For external use only, and also Shake the Bottle,' with the appropriate name, e.g. 'The Liniment,' 'The Cream,' or 'The Aplication.'

### COMPOUND ACACIA EMULSIONS

The emulsions in the two preceding exercises are simple, containing only one oily substance and the aqueous vehicle. Frequently emulsions are required to contain other substances and sometimes more than one oily liquid.

#### Emulsions Containing More than One Oily Liquid

When two or more oily liquids are present, the quantity of acacia required for each is calculated and the sum of these quantities is used for the emulsion.

#### Emulsions Containing Water-soluble Substances

Most of the substances included in emulsions are water soluble, e.g. salts, syrups, glycerin.

Water soluble substances are dissolved in, or mixed with, the aqueous vehicle required at Stage 3 for the approximate completion of the desired volume. In this way they are used in as dilute a state as possible—an important *desideratum*, because

### EXERCISE 5 1

#### Recipe—

Olei Olive	$\frac{3}{3}$
Aquam	$\frac{3}{4}$
Fiat mistura Signa Cochlearia medium bis in die sumendum	
Olive Oil is a fixed oil, and, applying the rules given (p. 56) the formula for the primary emulsion will be—	
Olive Oil	8 drachms
Powdered Acacia	2 drachms
Water	4 drachms
Complete Stages 1 and 2 of the <i>Method for Simple Emulsions</i> (p. 56). The approximate volume of the primary emulsion is 14 drachms; therefore use 18 drachms at Stage 3. Finally, adjust to volume in a measure.	

### EXERCISE 5 2

#### Recipe—

Olei Terebinthinae	8 ml
Aquam Cinnamomi	ad 40 ml
Fiat haustus Signa Statim capiendus	
Oil of Turpentine is a volatile oil and, applying the rules given (p. 56), the formula for the primary emulsion will be—	
Oil of Turpentine	8 ml
Powdered Acacia	4 G
Cinnamon Water	8 ml
Complete Stages 1 and 2 of the <i>Method for Simple Emulsions</i> (p. 56). The approximate volume of the primary emulsion is 20 ml, therefore use 20 ml of Cinnamon Water at Stage 3. Finally, adjust to volume.	

## EXERCISE 5 3

## Recipe—

Olei Morrhuae	5 i
Syrupi	5 iii
Ferri et Ammonii Citratis	5 ii
Aquam Cinnamomi	ad 5 iii

Fiat emulsio Signa Cochlearia parva duo bis in die capienda.

Cod liver Oil is a fixed oil, and, applying the rules on p. 56, the formula for the primary emulsion will be—

Cod liver Oil	8 drachms
Powdered Acacia	2 drachms
Cinnamon Water	4 drachms

Complete Stages 1 and 2 of the *Method for Simple Emulsions* as before. The volume of the primary emulsion is approximately 14 drachms, leaving approximately 10 drachms for completion of the product. Therefore dilute the Syrup to 10 drachms, dissolve the Iron and Ammonium Citrate in the mixture, and use the resultant liquid for Stage 3

some substances have de-emulsifying properties i.e. they might destabilize the emulsion if added in concentrated solution.

## Emulsions Containing Resinous Tinctures

When these tinctures are dispensed in mixtures, it is necessary in most cases to add a suspending agent, partly as a thickening agent, and partly as a protective colloid. On the other hand, such addition

is not necessary for emulsions containing resinous tinctures, because the gum present forms a protective colloid for those particles of resin precipitated in a colloidal state; further, the viscosity of the emulsion maintains the large particles in suspension.

The resinous tincture is added at Stage 3 to the primary emulsion after the latter has been diluted to equal the final volume, allowing for the volume of the tincture itself.

## EXERCISE 5 4

## Recipe—

Terebene	5 i ss
Olei Amygdale	5 iv
Tincturæ Tolutanæ	5 ij
Tincturæ Ipecacuanhae	5 ij
Aquam	ad 5 vj

Fiat mixtura Signa Cochlearie magnum quaque quarta hora capendum.

The emulsion contains Terebene and Almond Oil, and, as mentioned on p. 57, the quantity of Acacia needed is the sum of the amounts calculated separately for each oil. Hence the formula for the primary emulsion will be—

Terebene	1½ drachms
Almond Oil	4 drachms
Powdered Acacia, 45 gr + 60 gr =	105 grams
Water	210 minims

Complete Stages 1 and 2 of the *Method for Simple Emulsions* (p. 56). The volume of the primary emulsion is approximately 11 drachms, therefore dilute the Ipecacuanha Tincture with 33 drachms of water for Stage 3. Measure the Tincture of Tolu in a dry measure and pour into the centre of the emulsion whilst stirring rapidly. Finally, adjust to volume

### Emulsions containing Substances Insoluble in Oil or Water

Substances insoluble in either phase are occasionally prescribed, e.g. bismuth carbonate in a castor oil emulsion, and phenolphthalein in a liquid paraffin emulsion. These are dealt with as follows—

Carbonates and hydroxides of divalent metals, e.g. calcium carbonate, must be finely powdered, if necessary, and triturated with the pre formed primary emulsion to form a smooth cream, and the emulsion then completed in the usual way. Direct admixture of the un-emulsified oil with the carbonates or hydroxides of divalent metals must be avoided, because the latter may react with the free fatty acid present in all animal and vegetable oils, forming a divalent soap which, like other divalent soaps, promotes formation of a water-in-oil emulsion and thus opposes formation of the desired oil-in-water emulsion by the acacia.

Other insoluble substances may be finely powdered in a mortar (if not already in fine powder), and mixed with the acacia required for the primary emulsion. The oil is then added, and the primary emulsion prepared in the usual way. Exercise 57 illustrates the procedure.

### Emulsions Containing a Small Proportion of Oily Substances

With the exception of Exercise 54, the emulsions have hitherto contained one sixth or more of their volume of oily liquid. Generally speaking, when emulsions contain appreciably less than 10 per cent of oily liquid, and are prepared with the ordinary

proportion of acacia, they are somewhat unstable, and readily cream. For example, Solution of Calciferol may be prescribed for a young infant in doses of less than 0.25 ml with sufficient aqueous vehicle to produce 1 drachm of emulsion. This form is more acceptable than an oily liquid, and is miscible with bottle-milk. If the normal proportion of acacia is added the product will contain less than 4 per cent of oily liquid, and less than 1 per cent of acacia. In this dilution the emulsion would readily cream upon standing, and some of the oil globules might coalesce. Consequently it is necessary to add a sufficient quantity of a fixed oil (e.g. olive or almond oil) to raise the proportion of oily liquid to about 10 per cent of the finished product.

The following substances are often prescribed in an emulsion, with a dose representing appreciably less than 10 per cent of the finished emulsion, and may, therefore, need suitable dilution with a fixed oil before emulsification—

Bromoform	Concentrated Solution of Vitamin D
Vitamin A	Halibut liver Oil
Concentrated Solution of Vitamins A and D	Many Volatile Oils Solution of Calciferol

### ALTERNATIVE METHOD OF PREPARING ACACIA EMULSIONS

Acacia in the dry form has been used in making the preceding emulsions, the method is called the dry-gum method, and for small scale work it has ousted the older, wet gum method.

The rules for the proportion of oil, gum, and

### EXERCISE 55

#### Recipe—

Liquoris Calciferolis	m n
Glycerini	m v
Aquam	ad 3 i

Fiat emulsio Signetur More dicto danda Mitte 3 u

Each dose of Solution of Calciferol should be diluted to 6 minims with olive oil, i.e. to a volume representing 10 per cent of the finished emulsion. The formula for the primary emulsion is—

Solution of Calciferol	32 minims
Olive Oil	64 minims
Powdered Acacia	24 grains
Distilled Water	48 minims

Complete Stages 1 and 2 of the Method for Simple Emulsions (p. 56). The volume of the primary emulsion is approximately 3 drachms, therefore dilute the glycerin to 13 drachms, complete Stage 3 with the mixture, and then adjust to volume.

water for the primary emulsion have already been stated, and the method is as follows—

Triturate the powdered acacia with water to form a mucilage add the oil a little at a time, with constant and rapid trituration. Should the liquid in the mortar become 'ropy,' add a few drops of water and continue trituration until a homogeneous mixture is re formed. When all the oil has been added, continue trituration for a few minutes, and then complete the emulsion, following the usual procedure.

The method may be successfully used for very viscous substances. It is essential to use either a freshly-made mucilage, or a stock mucilage which has been stabilised immediately after preparation, by heating for 1 hour at 80°C.

The dry-gum method is quicker and, for dispensing purposes, gives better results.

*Agar Emulsions* (so called) Agar is obtained from certain algae and consists chiefly of a carbohydrate,

gelose. It may be obtained as a fine powder, or in strips, or in a shredded form—the last named being the most suitable form for administration or for the extemporaneous preparation of a solution.

Agar is insoluble, but swells slightly in cold water. It dissolves in boiling water, and, upon cooling, a solution containing 0.5 per cent or more sets to form a jelly. It is evident, therefore, that only a very small proportion of agar in solution can be included in a fluid emulsion—the presence of more than about 1 per cent would yield a solid or semi solid product.

Agar is not a good emulsifying agent, as it forms very coarse emulsions. On the other hand, the viscosity of the jelly prevents or retards coalescence of the oil globules. Hence agar may be classed with tragacanth, in that it minimises creaming of emulsions by increasing the viscosity. So-called agar emulsions are of necessity prepared with a suitable proportion of acacia or other emulsifying agent as in Exercise 5.6

### EXERCISE 5.6

Liquid Paraffin	2 oz
Phenolphthalein	30 gr
Agar	20 gr
Acacia	½ oz
Syrup	¼ oz
Cinnamon Water	to 6 oz

Make an emulsion Label Three teaspoonfuls to be taken twice a day

#### Method

Phenolphthalein is insoluble in water and oil; therefore, as mentioned on p. 59, it may be finely powdered and mixed with the acacia before adding the liquid paraffin. Make a primary emulsion in a hot mortar in the usual way, thus—

Liquid Paraffin	2 oz
Phenolphthalein	30 gr
Acacia	½ oz
Cinnamon Water	.1 oz

The volume of the primary emulsion is approximately 3½ oz. Therefore heat the agar (shredded) with 2 oz of distilled water\* in a tared dish over a small flame, until solution is effected, while hot, adjust it to 2 oz by weight add 24 minims of Concentrated Cinnamon Water (to convert the distilled water used to Cinnamon Water—if the latter were used initially most of the contained volatile oil would be volatilised), then add the Syrup, and mix. Gradually add the hot mixture to the warm primary emulsion with constant trituration, and transfer to a measure and adjust to volume. Finally, pass through a hand homogenizer in order to render the product whiter and less liable to cream.

\* This part of preparation should be started prior to preparation of the primary emulsion.

**EXERCISE 5 7**

Liquid Paraffin	2 oz
Phenolphthalein	20 grains
Agar	20 grains
Acacia	20 grains
Tragacanth	5 grains
Syrup	$\frac{1}{2}$ oz
Distilled Water	6 oz

Make an emulsion Label One tablespoonful to be taken at bedtime This formula differs from the previous one in that only a small quantity of acacia is present together with some tragacanth It is intended to be made with an emulsifying machine

A satisfactory emulsion can be made as follows—

Prepare a solution of agar as in the previous exercise Warm the liquid paraffin to about 60°C and triturate with it the acacia, tragacanth and phenolphthalein in a warm mortar Add the agar mucilage hot and triturate to mix thoroughly Add the syrup and pass through a hand homogeniser Pass sufficient water through the homogeniser to make the product up to volume

**Class 2 Irish Moss Emulsions**

The mucilage of Irish Moss for this purpose is prepared by washing the dried seaweed with water, digesting it in 40 times as much water by heating on a water bath and finally straining the mucilage through cotton wool in a hot water funnel It is

cheap and produces a very stable preparation which will stand for a long while without creaming It is used largely in making Cod liver Oil Emulsions but is not suitable for preparing small quantities of emulsions unless a hand homogeniser is used

**EXERCISE 5 8**

Cod liver Oil	2 oz
Creosote	10 minims
Glycerin	4 drachms
Mucilage of Irish Moss	3 oz
Water to	6 oz

Label The Cod Liver Oil Emulsion

**Method**

The Decoction of Irish Moss is made as described above and placed in an eight or ten ounce bottle The creosote is dissolved in the cod liver oil added to the decoction when it has cooled and shaken vigorously The glycerin is added and the coarse emulsion passed through a hand homogenising machine Finally the volume is adjusted with water These emulsions need a preservative e.g. about 1 in 500 of chloroform

**Class 3 Tragacanth Emulsions**

Tragacanth alone is not a good emulsifying agent it produces coarse emulsions and examined microscopically they reveal comparatively large globules

of oil It is chiefly used in conjunction with acacia to give increased viscosity to emulsions in order to prevent creaming (see p 71)

### Class 4 Cellulose Derivatives\*

Cellulose may be represented by the following formula—



where  $n$  may be about 1,000. The introduction of the methoxyl ( $-OCH_3$ ) or ethoxyl ( $-OC_2H_5$ ) groups results in the formation of methyl or ethyl ethers. In each of the anhydroglucose units there are three (OH) groups where methylation or ethylation can take place, but in the usual pharmaceutical product there are between one and two of these groups introduced. The wide variety among the products on the market is due to the possible variation in the extent of substitution and the variation in the length of the cellulose chain. Long chain compounds are more viscous.



Methylcellulose 450 B.P. contains between 26 and 32 per cent of methoxyl. The B.P.C. describes three forms of methylcellulose—

- Methylcellulose 20
- Methylcellulose 2,500
- Methylcellulose 4,500

The number indicates the approximate viscosity in centistokes of a 2 per cent mucilage.

The introduction of carboxymethyl groups ( $-CH_2COOH$ ) instead of methoxyl or ethoxyl groups gives rise to a series of compounds of similar type but with certain different properties. These are usually available as the sodium salt.



The average number of groups introduced per anhydroglucose unit is 0.7 in most pharmaceutical examples.

These cellulose derivatives are used as suspending

\* See also p. 151

thickening and emulsifying agents. They are also used in the preparation of dermatological jellies (See p. 151). Methylcellulose is soluble in cold water and insoluble in hot water. Mucilages may be prepared by one of the following methods:

- 1 Mix well with cold water and set aside for some hours, preferably overnight.
- 2 Mix well with cold water and hasten solution by using a mechanical stirrer.
- 3 Hydrate the powder by adding about one third of the water heated to boiling, then add the remainder of the water, ice-cold, and stir until homogeneous.

Sodium carboxymethylcellulose is soluble in cold water and more readily soluble in hot water.

Methylcellulose 20 is used in the preparation of Emulsion of Liquid Paraffin and Phenolphthalein B.P.C. Methylcellulose 20 forms neutral colourless mucilages without odour or taste which are stable over a wide pH range.

Reported incompatibilities are related to the grade of compound used, its concentration, and the concentration of the interfering substance. They are evidenced by turbidity, loss of viscosity etc. No complete picture can be presented but the following reported incompatibilities should be noted:

Methylcellulose - Phenol, resorcinol, tannic acid and silver nitrate.

Sodium carboxymethylcellulose - Phenylmercuric nitrate, phenol, tannic acid and the metallic ions—aluminium, iron, mercury, silver and zinc.

### PREPARATION OF EMULSION WITH METHYLCELLULOSE

These emulsions are not made by mortar and pestle methods but by shaking together the methylcellulose mucilage and the oil phase and passing the product through an emulsifying machine.

### EXERCISE 5.9

Methylcellulose 20	4.0 G
Liquid Paraffin	50.0 ml
Vanillin	0.1 G
Syrup	25.0 ml
Chloroform Water to	200.0 ml

Label "The Liquid Paraffin Emulsion."

#### Method

Add about 40 ml of boiling water to the Methyl-cellulose 20. Then add 62.5 ml of double-strength Chloroform Water in which the Vanillin is dissolved, and stir until homogeneous. Add the Syrup and the Liquid Paraffin and stir well. Pass the product through a hand homogeniser followed by the remainder of the water to adjust to volume.

**EXERCISE 5 10**

*Prepare 8 fluid ounces of Emulsion of Liquid Paraffin and Phenolphthalein B P C*

Follow the directions in the Codex and compare with the above directions. It is worth noting that this preparation was reported to develop lumpiness on storage. A report (1960) from the Department of Pharmaceutical Sciences of the Pharmaceutical Society states that this may be avoided if proper storage conditions are observed. It should be kept in well-closed, well filled containers in a moderately cool place.

**Class 5. Salts of Alginic Acid**

Sodium alginate forms very viscous solutions with water, and certain of these have been used with success as suspending, stabilizing and emulsifying agents. A 1 per cent solution forms a suitable mucilage for the preparation of emulsions. Sodium

alginate is incompatible with acridine derivatives, crystal violet, phenylmercuric nitrate, calcium salts (calcium alginate is insoluble in water), heavy metals and alcohol if over 5 per cent (See Exercise 5 11)

**EXERCISE 5 11**

Liquid Paraffin -	2 oz
Acacia -	35 grains
Sodium Alginate -	30 grains
Water -	8 oz

Label "The Paraffin Emulsion"

*Method*

Mix the Liquid Paraffin with the acacia, dissolve the sodium alginate in some of the water and add to the paraffin acacia mixture. Pass through a hand homogenising machine.

The solution of sodium alginate is made by adding water to it, with continual stirring and allowing the mixture to stand overnight.

These emulsions require a preservative, and sodium benzoate should be avoided, as it is reported to cause undue thickening of the sodium alginate mucilage.

**Class 6 Soap Emulsions**

These may be divided into two classes—

**(a) Soap Emulsions prepared with Soft Soap**

Soft Soap is used on unspecified prescriptions, in the following proportion—

For fixed and volatile oils One tenth as much Soft Soap as Oil

For fats One-fifth as much Soft Soap as Fat

the Soft Soap Gradually add the oil with trituration, to form a smooth suspension

**STAGE 2**

Place some water in a bottle. This quantity of water together with the soap should constitute about one third of the volume of oil. Gradually add the oily suspension, shaking the bottle after each addition.

**STAGE 3**

Add any fluid ingredients with the approximate quantity of water to produce the required volume. Strain through muslin and adjust to volume.

*Method***STAGE 1**

Finely powder any solid ingredients and mix with

**EXERCISE 5.12**

Prepare 100 ml of Turpentine Liniment B.P.

This liniment contains Oil of Turpentine, Camphor, and Water, with Soft Soap as the emulsifying agent.

Following the somewhat indefinite directions in the Pharmacopoeia often leads to failure in making this preparation. Therefore follow the above method thus—

Triturate the Camphor with the Soft Soap. Gradually add the Turpentine Oil to form a smooth suspension. Place about 15 ml of Purified Water in a bottle and add the oily suspension in small quantities, shaking after each addition. Strain through muslin and adjust to volume with Purified Water. The emulsion tends to become aerated, and after setting aside for some time the volume may be considerably less. Straining through muslin helps to remove air but it is wise to set the product aside for some time before finally adjusting to volume.

**(b) Soap Emulsions in which the Soap Is Formed by Interaction of the Free Fat or Resin Acid Present in the Oil, or Resin, with Some Alkaline Liquid Present**

All fixed oils contain a proportion of free fatty acid, although small, it is sufficient to form enough soap to emulsify the oil. Liquid Paraffin and Turpentine Oil do not contain any free acid, hence cannot be emulsified by means of alkaline substances unless a free fatty acid is added.

For external application, Calcium Hydroxide Solution is generally used in these emulsions. It should be remembered that it absorbs carbon dioxide from the atmosphere, so that, during careless or protracted storage, sufficient may be absorbed to precipitate a large proportion or even the whole of the calcium hydroxide as carbonate—the remaining liquid being water. Hence, if failure occurs in making an emulsion with Calcium Hydroxide Solution, the exercise should be repeated with fresh solution.

**METHOD FOR SIMPLE ADMIXTURES**

Shake the oil or oily mixture containing free fatty acid and the whole of the alkaline liquid vigorously

together until a cream is formed, then dilute with the aqueous vehicle (if any).

**METHOD FOR MORE COMPLEX ADMIXTURES**

**STAGE 1**

Place any dry ingredients in a mortar and powder if necessary.

**STAGE 2**

Mix together, by melting if necessary, the fatty and oily ingredients and triturate with the powder in the mortar.

**STAGE 3**

Add the alkaline liquid, and triturate briskly to form a cream.

**STAGE 4**

Add any liquid ingredients, and then adjust to volume.

Exercise 5.13 illustrates the Method for Simple Admixtures, while Exercises 5.14 and 5.15 indicate the Method for Complex Mixtures.

**EXERCISE 5.13**

*Recipe—*

Oleic Acid	1 ml
Dilute Solution of Ammonia	5 ml
Turpentine Oil	25 ml
Purified Water	to 100 ml

Label The Liniment To be rubbed in as directed.  
Follow the Method for Simple Admixtures, first mixing together the Oleic Acid and Turpentine Oil.

**EXERCISE 5.14***Recipe—*

Calaminae	1 G
Zinc Oxidi	1 G
Olei Olive	
Liquoris Calcii Hydroxidi	ana 15 ml

Fiat linimentum. Signetur Quotidie utendum

Follow the *Method for Complex Admixtures*, given above, thus—

- 1 Place the Calamine and Zinc Oxide in a mortar and mix.
- 2 Triturate with the Olive Oil
- 3 Add Calcium Hydroxide Solution, and triturate briskly until a cream is formed

**EXERCISE 5.15***Recipe—*

Zinc Oxidi	
Ichthammolis	
Adipis Lanæ	ana 5 ij
Olei Olivæ	5 x
Liquorem Calcii Hydroxidi	ad 5 ij

Fiat cremor Sigma Modo dicto utendus

Follow the *Method for Complex Admixtures*, thus—

- 1 Place the Zinc Oxide in a mortar
- 2 Melt the Wool Fat and Olive Oil together, and mix with the Zinc Oxide
- 3 Measure 1 oz of Lime Water (the approximate volume needed) add to the above, and triturate briskly
- 4 Add the Ichthammol adjust to volume with more Lime Water, and mix thoroughly

**(c) Organic Soap Emulsions**

Triethanolamine stearate and oleate are the commonest examples of this group of emulsifying agents. Triethanolamine,  $N(CH_2CH_2OH)_3$ , is a viscous liquid resembling glycerin as supplied commercially and is miscible with water. It usually contains a proportion of diethanolamine,  $NH(CH_2CH_2OH)_2$ , and monoethanolamine,  $NH_2(CH_2CH_2OH)$ . Like ammonia, which it resembles structurally, it combines with fatty acids to form salts. It is less alkaline than ammonia and its neutral soaps have a pH of about 8. It is thus less alkaline than the alkali soaps and more suitable for application to the skin but it is not suitable for preparations for internal use. The proportion of triethanolamine to the usual fatty acids is about 1 to 4 although usually an excess of acid is used to give greater stability to the resulting emulsion.

**PREPARATION OF EMULSIONS  
WITH TRIETHANOLAMINE SOAPS**

1 In dispensing, the triethanolamine soaps are usually formed *in situ* as in the case of ammonium

and calcium soaps already described. The triethanolamine is mixed with the water or aqueous solution and the fatty acid is mixed with the oils or fats, warming to melt and mix solid fats and acids. The aqueous solution is warmed to the same temperature and the oily solution added. The mixture is gently stirred until cool. Vigorous stirring promotes frothing and should be avoided.

2 The triethanolamine, acid, and water or aqueous liquid may be gently heated together to form a *soap solution*. To this warm soap solution the fats or oils are added at about the same temperature and the product stirred until cool.

3 The triethanolamine may be added to a solution of the fatty acid in oil or fat to produce a *self emulsifying oil*. An emulsion is readily formed when water is added to this mixture. Mixing is done when both phases have been brought to the same temperature.

Liquid fatty acids, such as oleic acid may be emulsified in the cold.

**EXERCISE 5.16**

Triethanolamine	15 minims
Oleic Acid	60 minims
Benzyl Benzoate	6 drachms
Water	4 fl. oz

Proceed according to Method I, no warming being necessary

**Class 7. Saponin Emulsions**

Quillaja liquid extract (and more rarely Tincture of Senega) is sometimes prescribed with oils as an emulsifying agent—usually when only one dose is required, in order to avoid the longer method with acacia. The emulsion is only temporary, and these tinctures should never be used in this capacity unless definitely prescribed. They both contain substances belonging to the group called saponins, which lower interfacial tension between oil and water, so that, when shaken with a mixture of oil and water, they

produce smaller globules, which consequently take longer to separate upon standing

*Method*

Place the oil and liquid extract of Quillaja in a bottle and shake vigorously. Mix or dissolve any other ingredients with the approximate quantity of aqueous vehicle required to complete the volume, and add this mixture a little at a time, shaking vigorously after each addition

**EXERCISE 5.17**

Prepare 10 fluid ounces of Emulsion of Peppermint B P C

Label as such

Follow the above directions

**Class 8 Starch Emulsions**

The use of mucilage of starch as an emulsifying agent is very limited, being usually restricted to preparations intended as *enemata*. Starch mucilage is not a true emulsifying agent—products made with

it are really suspensions of large oil globules, which are prevented from coalescing by the high viscosity of the starch mucilage

**EXERCISE 5.18***Recipe*—

Olei Ricini	5 ss
Amyli	5 ii
Aquam	ad 5 x

Fiat emulsio secundum artem Signa Enema, hora somni utenda

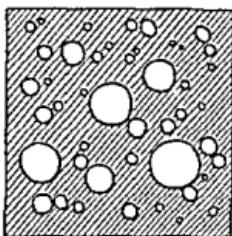
*Method*

Triturate the starch with sufficient water to form a smooth cream, transfer to a measure and add water to about  $9\frac{1}{2}$  oz. Place in a flask and heat to boiling with frequent agitation. The starch is gelatinized, and forms a mucilage. Remove from the flame and immediately hold the flask in a stream of cold water, rotating constantly, until cold—by this means, formation of a 'skin' is prevented. Then readjust the liquid to  $9\frac{1}{2}$  oz, add the Castor Oil, mix by vigorous stirring, and transfer to a bottle

**Class 9 Wool Fat Emulsions****Class 10 Beeswax Emulsions****Class 11 Synthetic Wax Emulsions**

These three classes of emulsions usually include emulsion creams of ointment like consistency and 'Ointment Emulsions' (p 135)

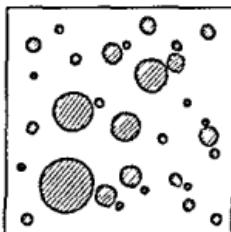
## TYPES OF EMULSIONS



(a)

## OIL-IN WATER EMULSION

This type of emulsion is produced by—  
Acacia and other Gums, e.g. Exercise 5 1  
Methylcellulose, e.g. Exercise 5 9  
Sodium Alginate, e.g. Exercise 5 11  
Alkali and Ammonium Soaps, e.g. Exercise 5 13  
Saponins, e.g. Exercise 5 17  
Synthetic Waxes, e.g. Exercises 10 11, 10 16, 10 18



(b)

Fig. 51 TYPES OF EMULSIONS

Shaded areas are aqueous

WATER-IN-OIL EMULSION  
(often called an 'invert' emulsion)

This type of emulsion is produced by—  
Wool Fat, e.g. Exercise 10 6  
Wool Alcohols, e.g. Exercise 10 8, Beeswax, e.g. Exercise 10 9, Metallic Oleates, i.e. Soaps of the non-alkali metals, e.g. Exercise 10 10

Table 52  
Tests for Identifying Emulsion Type

## 1 Dilution Tests

Oil in-water emulsions remain stable upon unlimited dilution with water, i.e. with the continuous phase or dispersion medium, but will not remain homogeneous upon the unlimited addition of oil—the latter will separate.

These facts may be confirmed by—

- (a) Stirring a few drops of acacia emulsion (e.g. Ex 5 1) with water perfect diffusion will take place
- (b) Stirring together equal volumes of a fixed oil and an acacia emulsion (e.g. Ex 5 1) the oil will quickly separate

## 2 Test by Staining Oil

Triturate together scarlet red (0.05 G) and olive oil (10 ml). Emulsify the oil with acacia and place a drop on a microscope slide, cover with a cover-slip, and examine under a microscope.

The dispersed globules will appear red, and the 'ground' colourless, proving that oil forms the disperse, and water the continuous phase—scarlet red being soluble in oil but not in water.

## 1 Dilution Tests

Water-in-oil emulsions remain stable upon unlimited dilution with oil, i.e. with the continuous phase or dispersion medium, but will not remain homogeneous upon the unlimited addition of water—the latter will separate.

These facts may be confirmed by—

- (a) Stirring a few drops of a 'lime' cream (e.g. Ex 5 14) with oil perfect diffusion will take place
- (b) Stirring together equal volumes of water and a 'lime cream' (e.g. Ex 5 14) the water will rapidly separate

## 2 Test by Staining Oil

Triturate together scarlet red (0.05 G) and olive oil (10 ml). Shake the filtrate with an equal volume of lime water and examine a drop of the resultant cream under a microscope.

The dispersed globules will be colourless, and the 'ground' will appear red, proving that water forms the disperse, and oil the continuous phase.

(continued overleaf)

Table 52 continued

### 3 Test by Electrical Conductivity

Water conducts electricity, hence an emulsion in which water forms the continuous phase acts as a conductor. Oil is a non-conductor, hence an emulsion in which oil forms the continuous phase acts as a non-conductor. A small low-striking voltage Neon Lamp, e.g. the Osglim Neon Lamp for 200-volt mains, is required for the test. It is connected in series with a bayonet fitting plug and two metal electrodes, e.g. two pieces of stout copper wire. When the plug is inserted into a lampholder and the electrodes touched together, the lamp will glow. If the electrodes are held apart and immersed in an oil in-water emulsion the lamp will also light. In a water in-oil emulsion the lamp fails to light, or flickers spasmodically. Some of the emulsions forming the exercises in the chapter, and subsequently some of the ointment emulsions, should be tested to verify their type.

## CONCENTRATION OF DISPERSE PHASE

It has been stated that the disperse phase consists of tiny globules or spheres, but this is not true if the emulsion is very concentrated. If small spheres of the same diameter are closely packed into a given space, they will not occupy more than about 75 per cent of the total capacity, however small the spheres. If the globules in an emulsion were quite spherical and all of the same size, it would be thus impossible to obtain more than 75 per cent of the total volume in

disperse phase. However, in emulsions the spheres are not all of the same size, nor always spherical, and, as a consequence, it is possible to prepare an emulsion containing more than 75 per cent of disperse phase. It is, however, difficult to prepare, by hand, emulsions which contain more than 75 per cent of disperse phase and this fact must be kept in mind in preparing formulæ and methods for making emulsions.

## FORMULATION

A knowledge of the formulation of emulsions can be successfully acquired only after considerable experience in the preparation of emulsions. Nevertheless, the following notes indicate the various factors involved and help to systematise an approach to the problems involved.

### Choice of Emulsifying Agent

In general dispensing practice it is not always necessary for the pharmacist to decide upon the emulsifying agent which should be used. On the other hand there is now a greater tendency than formerly, on the part of the prescriber, to leave such technical points to the specialised knowledge of the pharmacist, and he should make himself familiar with the numerous emulsifying agents available, their special properties, advantages and disadvantages.

At the beginning of this chapter a simple classification is given comprising the names of the better known emulsifying agents in general use, followed by typical examples of formulæ in which they are employed. Other examples are given in Chapter 10, and these should also be carefully noted.

For the extemporaneous preparation of emulsions for internal use for which no emulsifying agent is specified, by far the most popular and useful is Acacia. It can be easily stored in powder form

without decomposition, it is colourless and odourless and it is, furthermore inert. In addition, excellent emulsions can be prepared with it on a small scale when the necessary skill is acquired. Exercises 51 to 57 illustrate its general usefulness.

A wide range of emulsifying agents for the production of both oil in-water and water in-oil emulsions is included in the exercises in this chapter and under 'Ointment Emulsions' in Chapter 10. It will be noticed that emulsions for external use are of both oil in water and water in-oil types, whereas emulsions for internal use are almost invariably of the oil in water type.

Although so many emulsifying agents are available it should be remembered that the choice is considerably narrowed by factors such as the purpose for which the emulsion is required, the presence of substances incompatible with certain emulsifying agents, the consistency of the final product etc. Moreover, it must be borne in mind that generally several different emulsifying agents might be equally suitable.

### Hand made and Machine-made Emulsions

Exercises in this book refer mostly to emulsions made by hand. It should be recognised that machine-made emulsions usually require considerably less

emulsifying agent Emulsions of the BPC are formulated for small scale work, and manufacturers using homogenisers and other emulsifying machines must modify the quantities of emulsifying agent in order to prepare emulsions of similar quality Compare the emulsions in Exercises 5.6 and 5.7

### Complementary Emulsifying Agents

Often it is preferable to use more than one emulsifying agent to produce the most satisfactory result This is well illustrated by the Acacia emulsions in which additional emulgents and stabilisers are commonly used For example, exercises are included in this chapter in which Tragacanth, Agar and alginates are used with Acacia Cellulose derivatives, pectin and other stabilising agents are similarly used (See Creaming of Emulsions, p 71)

Reference is made later to the use of traces of soap with glycoesters (p 139) Emulsifying Wax, it should also be noted, is a mixture of two emulsifying agents Similarly, quaternary ammonium compounds are used in combination with cetyl and stearyl alcohols In each of these the two emulsifying agents which are combined will produce emulsions of opposite types if used separately, but when combined produce very stable oil in water emulsions This is the principle elucidated by Schulman and Cockbain (see p 138) Mixtures of various non-ionic emulsifying agents are also used with success to produce stable products (see p 139)

### The Use of Preservatives

In selecting preservatives to combat bacterial and fungal attacks the following factors should be borne in mind—

- 1 The preservative should be compatible with the other ingredients of the emulsion
- 2 It should be non toxic
- 3 It should be tasteless (for internal emulsions), odourless and colourless

In aqueous emulsions and creams, complete freedom from contamination from micro organisms is not usually practicable but gross contamination can be prevented by the use of suitable preservatives Fungi are particularly to be guarded against since they multiply at and around room temperature in aqueous fluids They are, moreover, capable of growth over a fairly wide range of pH The following are commonly employed in pharmaceutical preparations, including liquid emulsions and creams

### BENZOIC ACID

Benzoic acid is permitted as a preservative by the Ministry of Food and is non toxic in the concentra-

tions used Its effect depends on the undissociated molecule and it is, therefore, less effective in alkaline or neutral media and most effective at pH values less than 5 In concentrations of 0.1 per cent it is used in certain BNF infant mixtures Mucilages may be preserved by a concentration of 0.2 per cent In Emulsion of Liquid Paraffin with Phenolphthalein it is used in a concentration of 0.11 per cent together with Chloroform 0.1 per cent

### SODIUM BENZOATE

Sodium benzoate is much more water-soluble than benzoic acid and is sometimes used as a preservative instead of benzoic acid It is, however, only effective in acid solution It is included in Emulsion of Liquid Paraffin in a concentration of 0.5 per cent with Chloroform 0.25 per cent

### ESTERS OF PARA-HYDROXYBENZOIC ACID

Certain of these esters are valuable preservatives The methyl and propyl esters are commonly used and are more effective and less toxic than benzoic acid For aqueous creams suitable concentrations are 0.05 per cent (methyl ester) and 0.2 per cent (propyl ester) The methyl ester is soluble in 500 parts of water and in 40 parts of warm vegetable oils The propyl ester is only slightly soluble in water and is soluble in 40 parts of vegetable oils

### CHLOROCRESOL

Chlorocresol is used in a strength of 0.1 per cent as a preservative in aqueous creams Its effectiveness in these creams, however, depends on its distribution between the aqueous and oily phases and is therefore, affected by the phase volume ratio Although 0.1 per cent is commonly used in these preparations, and would appear to be effective, its concentration in the aqueous phase will vary according to the partition coefficient between oil and water, and this should be considered in formulation

It should also be remembered that phenolic preservatives are incompatible with Cetomacrogol Emulsifying Wax and other emulsifying agents containing chains of oxyethylene groups (See p 141)

Elsewhere its use as a bactericide in injections is described

### CHLOROFORM

Chloroform is a commonly used preservative for extemporaneously dispensed preparations Chloroform Water (0.25 per cent v/v chloroform) is used as the vehicle in many mixtures, and in addition to its preservative properties it has a sweet taste and a

carmunative action. It is used (0.25 per cent) in Emulsion of Liquid Paraffin with benzoic acid.

### Antioxidants

Animal and vegetable fats and oils are subject to oxidation by atmospheric oxygen and by the enzymes associated with micro-organisms. Preservatives such as those mentioned above deal with the latter cause of oxidation. Antioxidants prevent oxidation due to atmospheric oxygen. These are described in Appendix I together with the usual percentages used.

### Degree of Stability Required

Dispensed emulsions which are to be used within a few days are not subjected to such a severe test of stability as those which may be stored for a prolonged period, possibly under varying conditions of temperature. The latter must, therefore, be formulated with very great care to ensure permanence. This will usually necessitate the use of thickening agents to increase the viscosity of the continuous phase and the homogenisation of the emulsion to reduce globule size (see 'Creaming of Emulsions' p. 71).

Coarse emulsions are useful for use as enemas, which are commonly oil-in-water emulsions in which starch is used as emulsifier.

Sometimes emulsions are required which break and leave a film on the skin to act as a protective. These are exemplified by barrier creams.

### Phase Volume Ratio

The concentration of the disperse phase (see pp. 59, 68) has an important bearing on stability. A disperse phase concentration of 30 to 60 per cent provides the most stable range. Higher concentrations of disperse phase, especially 75 per cent and over, may be troublesome to incorporate and may even result in phase reversal (see p. 75). Lower concentrations of disperse phase result in emulsions which tend to cream more readily.

### Antagonistic Substances

Substances which affect the emulsifying agent may cause instability. Electrolytes may also cause trouble. For example, calcium salts may cause phase reversal in an emulsion where the emulsifying agent is an alkali or a triethanolamine soap. Soap emulsions are broken by acids because the fatty acid is precipitated.

Some emulsifying agents are not stable over a very wide range of hydrogen ion concentration. Their effective range should be noted. Cationic emulsifying agents are incompatible with anionic emulsifying

agents. Non ionic emulsifying agents are stable to considerable concentrations of electrolytes.

### Flavours

These are selected by trial and error. As examples, vanillin for liquid paraffin emulsions and essential oil of almonds for cod liver oil emulsions are the flavours of common choice.

### FORMULATION BY THE H L B METHOD

A useful method for calculating balanced mixtures of emulsifying agents to provide a desired type of emulsion was first worked out by Griffin (1949-1954). This is called the Hydrophilic-Lipophile Balance or H.L.B. Method. The method is mainly of value for non ionic emulsifying agents and has been largely developed in connexion with emulsifying agents produced by The Atlas Powder Company (U.S.A.), who publish guides to formulation (1954), (1958).

In this method each emulsifying agent is given a number on the H.L.B. scale, high numbers indicating hydrophilic (water soluble) properties and low numbers indicating lipophilic (oil-soluble) properties. Emulsifying agents with high numbers will produce oil-in-water emulsions and those with low numbers will produce water-in-oil emulsions.

Table 5.3  
H.L.B. Ranges and their Applications

H.L.B. range	Application
3-6	W/O Emulsifier
7-9	Wetting agent
8-18	O/W Emulsifier
13-15	Detergent
15-18	Solubiliser

The H.L.B. scale was originally arrived at by experimentation by Griffin (1949), but more recently equations have been developed by Griffin (1954) by which the values can be calculated for many non ionic compounds. The values for a few selected emulsifying agents is given in Table 5.5 (Griffin, 1954).

Oils, fats and waxes used in emulsions require emulsifying agents of a suitable H.L.B. value to ensure satisfactory emulsification. For these oily materials, therefore, required H.L.B. values have been worked out according to whether oil in water or water in oil emulsions are required. (See Table 5.4.) When several oils and fats are included in a formula the total 'required H.L.B.' value may be

calculated and a suitable blend of emulsifying agents decided upon accordingly.

The following example of an emulsified lotion from the Atlas Powder Company publication (1954) illustrates the procedure.

Table 54

Some 'Required H L B' Values of oils and waxes (Atlas Powder Company, 1958)		
	W/O	O/W
Cetyl Alcohol	—	15
Lanolin, anhydrous	8	10
Mineral Oil, heavy	5	12
Mineral Oil, light	5	12
Beeswax	4	12
Soft Paraffin	5	12

Table 55

Some H L B Values of Emulsifying Agents	
Glyceryl Monostearate (Pure)	3.8
Glyceryl Monostearate, Self-Emulsifying (Tegin)	5.5
Sorbitan mono-oleate (Span 80)	4.3
Sorbitan mono stearate (Span 60)	4.7
Polyoxyethylene sorbitan mono-oleate (Tween 81)	10.0
Polyoxyethylene sorbitan mono-stearate (Tween 61)	9.6
Triethanolamine oleate	12.0
Potassium oleate	20.0

The proposed formula is—

Mineral Oil	35%
Lanolin	1%
Cetyl Alcohol	1%
Emulsifier	7%
Water	56%

'Required H.L.B.' values for the first three ingredients (Table 54) are respectively 12, 10 and 15. The percentage of the oil phase is 37% (35% + 1% + 1%) and the proportions of each are—

Mineral Oil	$\frac{35}{37} = 94.6\%$
Lanolin	$\frac{1}{37} = 2.7\%$
Cetyl Alcohol	$\frac{1}{37} = 2.7\%$

Total 'required H L B' value will, therefore, be—

$$\begin{array}{ll} \text{Mineral Oil} & 94.6 \times 12 = 114 \\ \text{Lanolin} & 2.7 \times 10 = 0.3 \\ \text{Cetyl Alcohol} & 2.7 \times 13 = 0.4 \\ \hline \text{Total 'required H L B'} & = 121 \end{array}$$

Various blends of emulsifying agents within this range are tried in order to narrow the range. A mixture of Span 80 (sorbitan mono-oleate) (H L B value 4.3) and Tween 80 (polyoxyethylene sorbitan mono oleate) (H L B value 15) in a ratio of 30 to 70 gives satisfactory results. The H L B value of this mixture is calculated as follows—

$$\begin{array}{ll} \text{Span 80} & 30\% \times 4.3 = 1.3 \\ \text{Tween 80} & 70\% \times 15 = 10.5 \\ \hline \text{H L B value of the mixture} & = 11.8 \end{array}$$

The possible combinations of the numerous non-ionic emulsifying agents is legion but formulation is much simplified by a working knowledge of this method.

### CREAMING OF EMULSIONS

It is well known that a layer of cream forms on the surface of milk when set aside for a few hours. This cream contains a much higher proportion of fat globules than the original milk, hence creaming may be defined as the formation of a layer of relatively concentrated emulsion.

An emulsion which has creamed may be rendered homogeneous again by shaking or stirring. Creaming is, however, undesirable in emulsions because the closeness of the oil globules in the 'cream' may favour the breakdown of the interface, followed by coalescence of the oil globules into a layer. In extreme cases the cream may separate almost completely,

leaving a comparatively clear aqueous layer beneath. This is very undesirable, because inaccurate dosage is very probable unless shaking is vigorous and prolonged.

To prevent creaming it is necessary to examine the influencing factors, the chief being the rising of globules of emulsified oil or fat to the surface of the emulsion. The rate at which an isolated globule will rise in a watery medium is governed by Stoke's law, which may be expressed as follows—

$$v \propto \frac{r^2(d_1 - d_2)}{n}$$

where  $v$  = rate of creaming,

$r$  = radius of globule,

$d_1$  = density of continuous phase,

$d_2$  = density of disperse phase,

$n$  = viscosity of dispersion medium

Stoke's law therefore formulates that—

- 1 *The rate is directly proportional to the difference in specific gravity between the oily and the watery phases*

Creaming could thus be minimised by making the specific gravity of the two phases equal. Most oils have a specific gravity below 1.00, so that it would be necessary to add alcohol (or some liquid of similar specific gravity) in order to lower that of the aqueous phase. This course is open to numerous objections—being costly and therapeutically undesirable.

- 2 *The rate is directly proportional to the square of the radius of the globule*

It will be apparent that, following the above law, small globules will rise less quickly than large. Consequently the tendency to cream may be minimised by finely subdividing the oil globules into minute uniform size.

An emulsion, whether made mechanically or by hand, will contain globules of different size, some quite small and others relatively large. Consequently further treatment is necessary to obtain uniformity of size, and this is effected by a machine called a homogeniser (see p. 74).

- 3 *The rate is inversely proportional to the viscosity of the dispersion medium*

Since an increase in temperature reduces viscosity and, therefore, encourages creaming, emulsions should be kept in a cool place.

A low disperse phase volume ratio also causes creaming (see p. 59).

From the above it will be seen that creaming may be minimised by increasing the viscosity of the emulsion.

Syrup and Glycerin, which are often included in emulsions as sweetening agents, increase the viscosity, but they also increase the difference in specific gravity between the aqueous and oily phases. Consequently, these substances are ineffective to prevent creaming.

On the other hand, Tragacanth and Methylcellulose increase viscosity without appreciably affecting the specific gravity; they are, therefore, included in many emulsions to raise the viscosity and thus retard creaming.

To summarise, the perfect emulsion is produced by—

- (a) Emulsifying the oil with the required quantity of emulsifying agent
- (b) Thoroughly subdividing the oil globules in a homogeniser to minimise creaming
- (c) Further reducing the tendency to cream, by including a thickening agent, e.g. Tragacanth or Methylcellulose
- (d) Storing the emulsion in a cool place

The formulae for the Acacia emulsions in the preceding practical section have not usually included the thickening agents in (c) because these exercises are regarded as practice in extemporaneous dispensing for more or less immediate consumption—in these circumstances the tendency to cream will not often be encountered, provided the instructions are carefully followed.

The use of thickening agents (c) and, similarly, the mechanical subdivision (b) are more in the domain of large scale manufacture, where the product may be stored a considerable time before use. The term 'shelf life' is sometimes applied to the period of storage.

## EMULSIFIERS

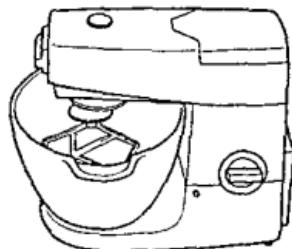
This chapter deals with the making of emulsions extemporaneously. This has generally meant 'by hand' as opposed to 'by machinery'. There is an unanswered argument for using a machine if it makes a more successful product and the process is time-saving. The following examples are illustrative of the types of small-scale emulsifiers. Larger scale apparatus is described in *Tutorial Pharmacy*.

In large-scale manufacture emulsions are usually prepared by the wet gum method (p. 59) because with an emulsifier it is simpler than the dry gum method. Additionally, with an emulsifier it is

cheaper because the mucilage is prepared by dissolving the tear form of Acacia in water, and thus the cost of powdering the gum is eliminated. Further a 'cleaner' emulsion ensues because the mucilage can be strained free from foreign matter before use, whereas its removal from the powder is impossible.

### KENWOOD MIXER

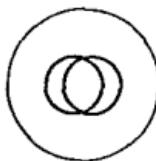
A really versatile mixing machine the Kenwood Mixer, Fig. 52, has many household uses but it is suitable for making small batches of emulsions. Its mixing action is particularly efficient since, as the



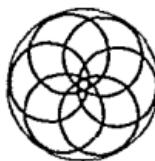
*Fig 5.2 THE KENWOOD MIXER*  
(Courtesy Kenwood Manufacturing (Woking) Ltd)

beaters rotate the axis on which they are fixed rotates also. This gives a planetary action (Fig 5.3) which reaches all the liquid in the mixing vessel and not just the centre portion which is the case with ordinary stirrers. Several types of beaters and whisks are available with the machine and these are quickly exchangeable by a simple locking device.

**SILVERSON MIXER EMULSIFIER (Laboratory Model)**  
This machine (Fig 5.4a) consists of an emulsifying head containing rotor blades (b) within a fine mesh sieve band (c). The head is suspended on long rods so that it may be immersed in the liquids to be emulsified. The blades in the head are rotated by a central rod driven by a small motor. In use the liquids are



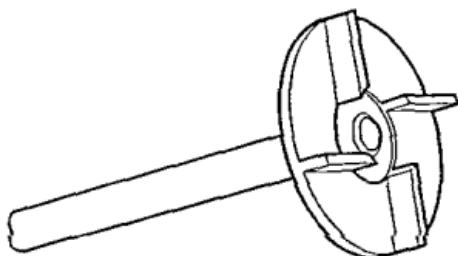
Ordinary mixing



Planetary mixing

*Fig 5.3 MIXING ACTION OF BEATERS*

sucked into the base of the emulsifying head and subjected to the vigorous mixing action of the rotor blades in the confined space. The liquid is continuously sucked in and expelled with considerable force against and through the sieve band. This sucking in and forcing out of the liquid sets up a circulation the pattern of which depends on the shape of the mixing vessel and effectively brings all the liquid through the head repeatedly. This repeated passage through the emulsifying head results in efficient breakdown of large globules. If necessary the mixing vessel may be jacketed so that the process can be carried out at different temperatures. Silverson mixers are made in many sizes. The laboratory model is suitable for batches of up to 2 gallons. Machines working on the same principle are made to process batches of 2 000 gallons.



(b)

(c)

*Fig 5.4 THE SILVERSON EMULSIFIER  
(LABORATORY MODEL)*  
(Courtesy Silver Machines Ltd)

## HOMOGENISERS

A considerable proportion of the oil globules in emulsions made with a mortar and pestle or in an emulsifier are large, often  $30 \mu^*$  or more in diameter. As mentioned on p. 72, sub-division of the oil globules is an important means of reducing creaming. Further, homogenisation, by reducing the size of the globules and thus increasing the surface area of the oil, raises the viscosity of an emulsion. Thus, when an emulsion is to be homogenised, less gum is needed to produce normal viscosity and in consequence homogenisation combines efficiency with economy of emulsifying agent.

All homogenisers embody the same principle, namely that of forcing the emulsion through fine interstices formed by closely approximated metal surfaces, and this principle has been applied in many different ways. Several of the small hand-driven machines, although invented and marketed for the preparation of synthetic cream from salt-free butter and milk, are admirably suitable for small-scale homogenisation of pharmaceutical emulsions. (Fig. 5.5) is a machine described below.

\*  $\mu$  = micron, the one thousandth part of a millimetre.

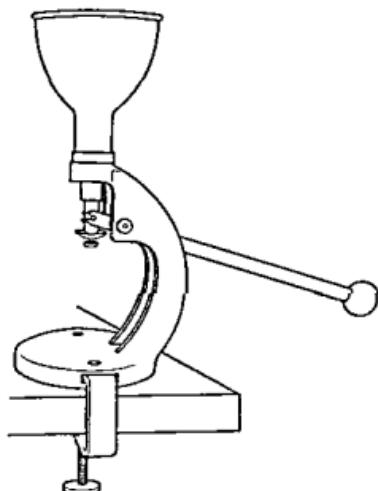


Fig. 5.5 THE Q.P. EMULSIFIER  
(Courtesy Ormerod Engineers Ltd., Rochdale)

### THE Q.P. EMULSIFIER

This homogeniser makes use of the well known principle that when a jet of water is forced under pressure against a wall, the jet is split into fine droplets. The pre-formed and more or less coarse emulsion is placed in the container shown in the upper end of Fig. 5.5, and pumped through by the handle on the right. The pump forces the emulsion through a fine aperture on to a baffle, thereby reducing the size of the oil globules. This reduction in size increases with the speed of pumping.

### Size of Globules in Emulsions

The size of the globules in an emulsion may vary from  $1 \mu$  (or rather less) to  $30 \mu$  or more in diameter. In an emulsion made either by hand or by a mechanical mixer, the globules range from  $1\text{--}50 \mu$ , but effective homogenisation will reduce their diameter to  $1\text{--}3 \mu$ .

It might be considered at first sight that an emulsion was a good one if the measurement of several hundred globules ranged from, say,  $1\text{--}3 \mu$ , and only a few exceeded  $15\text{--}20 \mu$ . The size-frequency based on the diameter of the globules is not, however, a criterion of quality, and to assess the degree of emulsification it is necessary to discover what proportion of the total volume of oil is present in small globules. If this proportion is high, the emulsion is a good one, but, if low, it is poor. For illustration—

Assume that measurement of the diameter of 100 oil globules in an emulsion showed that—

75	ranged from	$1\text{--}5 \mu$ (averaging $3 \mu$ )
10	" "	$5\text{--}10 \mu$ ( " $8 \mu$ )
15	" "	$10\text{--}30 \mu$ ( " $20 \mu$ )

The relative proportion of oil represented by these three size-frequency ranges is—

oil globules (average)	occupy†
dia	(cu $\mu$ )
75	$3 \mu$
10	$8 \mu$
15	$20 \mu$
Total cubic space occupied	= <u>66,598</u>

Therefore 75 per cent of the globules occupy  $\frac{1,061}{66,598}$  = (approx.)  $\frac{1}{63}$  or  $1\frac{1}{3}$  per cent of the volume of the oil.

† For simplicity in calculation, the globules are here regarded as true spheres with a volume  $\frac{4}{3}\pi r^3$  where  $r$  = radius of the sphere.

Hence, although 75 per cent of the globules are small in diameter, only  $\frac{1}{2}$  per cent of the volume of the oil is present in small globules

It follows therefore, that in comparing emulsions, or deciding whether they are good or poor, it is of no use to consider the proportion of globules with a small diameter—the criterion is how much of the total volume of the oil is present in this form, which is dependent upon the cube of the diameter

In general, an emulsion cannot be considered satisfactory unless about 50 per cent of its volume of oil is present as globules measuring less than  $5\text{ }\mu$  in diameter

The degree of subdivision of the oil in an emulsion may be determined thus—

- By measuring the diameter of several hundred oil globules by means of a micrometer, grouping and then calculating the volume of oil which each size-frequency group represents, as shown above
- By using a Haemocytometer This method was introduced by E. L. Smith and N. Grinling (1930) to supersede method (a), which is lengthy and tedious A description appears in the original papers

#### Phase Inversion by Antagonism of Emulsifying Agents

Soaps of divalent metals, e.g. calcium oleate, are water in oil promoting while soaps of monovalent

metals or ammonia form emulsions of the oil in-water type A mixture of such antagonistic emulsifiers added to oil and water would have no uniform emulsifying effect—at some points in the liquid an oil in-water emulsion might be formed, at others, the reverse But this unstable emulsion would be completely converted into an oil in-water emulsion by adding to it a small proportion of potassium oleate conversely, the addition of calcium oleate would stabilise the emulsion into the water in oil type Either addition will upset the ‘balance’, and the type of emulsion thus formed will depend upon the nature of that agent present in excess

The character of an emulsion made with a soap can, therefore, be changed by adding excess of a metallic oleate, and vice versa, and this is known as a Phase Inversion It is not confined to soaps and metallic oleates—sometimes it will be found with other pairs of substances which produce emulsions of differing types For example, beeswax yields a water in oil emulsion (e.g. Exercise 10.9), but the addition of soap inverts the emulsion

The above facts provide the explanation why a bottle which has contained a ‘lime cream’ can be cleansed with soap and water, but not with water alone The soap inverts the emulsion to the oil-in water type, thereby rendering it miscible with water

### CRACKING OF EMULSIONS

The expression ‘cracking’ of emulsions implies separation of the disperse phase as a layer It may be brought about in several ways—

#### 1. By Addition of an Emulsifying Agent of Opposite Type

As already noted, the soaps of monovalent metals produce oil in water emulsions, and the soaps of divalent metals produce water in oil emulsions It is evident that the addition of a carefully balanced quantity of sodium soap to a lime-soap emulsion will unstabilise the emulsion and lead to separation of the disperse phase as a layer Similarly anionic and cationic emulsifying agents are antagonistic

#### 2 By Decomposition or Precipitation of the Emulsifying Agent

Soft soap (principally potassium oleate) is decomposed by mineral acids forming oleic acid and a potassium salt of the mineral acid, neither of which has emulsifying properties Hence the addition of a mineral acid (and certain organic acids e.g. acetic acid) to a soft soap emulsion (e.g. Turpentine

Liniment) causes cracking, the oleic acid forming part of the oily layer and the potassium salt of the mineral acid dissolving in the aqueous layer

Sodium soaps are precipitated (salted out) by the addition of sodium chloride and certain other electrolytes, hence the addition of sufficient electrolyte to a sodium soap emulsion causes cracking by precipitating the emulsifying agent A similar effect is produced by the addition of certain electrolytes to emulsions prepared with potassium soaps—thus, if sufficient sodium chloride is added, most of the potassium soap is converted to sodium soap, and the latter is then precipitated

Acacia and other gums and also most proteins are insoluble in alcohol hence the addition of sufficient alcohol to emulsions prepared with these substances causes precipitation of the emulsifying agent, and cracking ensues

#### 3 By Addition of a Common Solvent

The addition of a liquid in which the disperse and continuous phases are soluble ‘destroys’ an emulsion by forming a one phase liquid, and this may be

regarded as a special case of cracking. To illustrate, oil of turpentine (or castor oil), water, and soft soap are all soluble in alcohol, hence the addition of sufficient alcohol to an emulsion of these substances will produce a clear solution.

#### 4 By Extremes of Temperature

This is chiefly of importance in the case of stored emulsions which may be kept for prolonged periods before being used. High temperatures reduce viscosity and encourage creaming. An emulsion which has creamed badly is more liable to crack than one which remains homogeneous. Over long periods even slightly raised temperatures may prove serious. Low temperatures are less likely to cause trouble unless freezing takes place, when ice may separate.

#### 5 By Micro-organisms

Unless suitably preserved an emulsion may develop moulds and bacteria. This could cause changes resulting in cracking but even if the emulsion did not crack it would be rendered unfit for use.

#### 6 By Creaming

It should also be remembered that creaming encourages cracking and, therefore, factors which cause creaming may lead to cracking.

#### REFERENCES

- ATLAS POWDER CO (1954) *A Guide to Cosmetic and Pharmaceutical Formulation with Atlas Products*
- DEPARTMENT OF PHARMACEUTICAL SCIENCES (1960) Liquid Paraffin Phenolphthalein Emulsion B P C, *Pharm J* 185, 519
- GRIFFIN, W C (1949) Classification of Surface Active Agents by H L B, *J Soc cosmet Chem* 1, 311
- GRIFFIN, W C (1954) Calculation of H L B values of Non Ionic Surfactants *ibid.* 5, 249
- SMITH E L and GRINLING, G N (1930) Studies in Emulsification Part I *Quart J Pharm Pharma col* 3, 354-361
- SMITH, E L and HASLEY V (1930) Studies in Emulsification Part II *ibid.* 3, 362-372
- SPLATON L M (1956) *Pharmaceutical Emulsions and Emulsifying Agents* *Chemist and Druggist*

#### REVISION EXERCISES 1 Recipe—

Olei Hypoglossi	m iii
Glycerini	m v
Aquam Cinnamomi	ad 3 i
Fiat mistura Signa Tertius horis capienda	Mitte 3 ii

#### 2 Recipe—

Cresosoli	m xxx
Olei Menthuae	3 i
Olei Cinnamomi	m v
Sodii Hypophosphitis	
Calcii Hypophosphitis	ana gr xv
Syrupi	3 iii
Aquam	ad 3 iii

Fiat emulsio Signa Cochlearia parva duo ter in die post cibos sumenda

#### 3 Prepare 100 ml of Emulsion of Magnesium Hydroxide and Liquid Paraffin B P C

4	Liquid Paraffin	33
	Cinnamon Water	to 100

Send 3 oz. Label Emulsion of Liquid Paraffin 33%

#### 5 Recipe—

Olei Ricini	3 i
Mucilaginis Acaciae	3 ii
Aquam Cinnamomi	ad 3 iv

Fiat emulsio Signa Cochlearia magnum quater in die capendum.

**SUGGESTIONS FOR PRIVATE STUDY**

- 1 Discuss briefly the object of combining Acacia and Tragacanth in formulæ for emulsions
- 2 Discuss the factors which influence the stability of an emulsion
- 3 Define the terms 'creaming' and 'cracking' used in reference to emulsions, and briefly explain how the former may be minimised, and how the latter may be brought about
- 4 Describe three methods by which the type of an emulsion can be determined
- 5 Discuss 'disperse phase concentration' in relation to the preparation of emulsions
- 6 Explain, with examples, what is meant by 'phase inversion' in connexion with emulsions



## 6

## Powders

THE exercises included in this chapter are based upon the following classification of powders—

- Class 1 Simple and Compound Powders
- Class 2. Powders enclosed in Cachets or Capsules
- Class 3 Compressed Powders (Tablet Triturates)
- Class 4 Granular Effervescent Powders
- Class 5 Powders for External Use

### PACKING OF POWDERS

Powders may be wrapped or bulked

#### (a) Wrapped Powders

In this form each dose is enclosed in paper. With fewer than six, it is usual to send them in an envelope, but for larger quantities boxes are preferred. They are wrapped in the following manner—

- 1 Select a box to hold the number required—stock patterns hold 6, 12, 24, etc. In the smallest size the powders are usually placed flat, but in the larger sizes they are placed on edge
2. Cut the required number of powder papers to the following dimensions—

Length  $\frac{1}{2}$  in less than twice the inside length of box

Width Three times inside width (or depth) of box.

- 3 Turn up one edge of each paper to one-seventh of its width, lay it with its turned up edge away from the dispenser, and each paper slightly overlapping the next

- 4 Place the balance so that the pillar is in a line with the left-hand edge of the spread papers, and place the material behind the papers to the right of the balance. The left hand is used for raising the beam

and the right hand for transferring the powder. Weigh out the powders, and place each centrally on the paper, beginning from the right and working inwards

5 Insert the near edge of the paper beneath the turned up edge, fold both over again—this will bring the fold to the centre. Turn the ends under by placing symmetrically over a powder folder made slightly less than the inside length of the box. Make the folds firm by smoothing them with a flexible spatula. Stack the powders in pairs, flap to flap, and place a rubber band around, finally box

*Double-wrapping.* The white demy used for ordinary powders is not suitable for volatile or hygroscopic powders unless lined with waxed paper. This is cut slightly smaller each way, and it is quite satisfactory to fold both papers at once. Double wrapping is necessary for powders containing—

Volatile Substances	Hygroscopic Substances
Camphor	Citric Acid
Chlorbutol	Iron and Ammonium Citrate
Menthol	Pancreatin
Thymol	Pepsin
Volatile Oils	Potassium Citrate
	Sodium Chloride

#### (b) Bulked Powders

These are usually sent out in screw-capped jars or bottles, with a mouth sufficiently wide to take the teaspoon or tablespoon, as directed. Quantities of powders in teaspoonsfuls and tablespoonsfuls vary widely and in many cases the dosage may not be critical. When, however, an accurate weight of powder must be taken a small pill box may be cut down to hold just the correct amount

## Class 1. Simple and Compound Powders

A simple powder contains but one ingredient, a compound powder, two or more. For simple powders the material is reduced to fine powder and weighed as directed above.

## Quantity of Material for Compound Powders

Loss is unavoidable for many reasons and, when mixing the bulk, the following rules should be observed—

- (i) If practicable, weigh out material for one powder more than required
- (ii) If this produces quantities containing a fraction of a grain not directly weighable, calculate for sufficient extra powders to produce directly weighable amounts
- (iii) If the total weight of each powder includes a fraction of a grain, add the calculated amount of lactose to make each powder directly weighable
- (iv) If the powder contains a fluid volume, the weight of which is unknown, adjust the mixed material by the addition of lactose, so that each powder is directly weighable

## METHOD FOR COMPOUND POWDERS

## STAGE 1

Separately powder a slight excess of the ingredients which are crystalline

## STAGE 2

Weigh out the required amounts of each ingredient, and of lactose, if necessary. Dilute the ingredient of smallest amount with the others, using them in ascending order of weight, and adding them in quantities which will double the weight of the mixture by each addition (Example 6 1)

## STAGE 3

Weigh out the prescribed number of powders, and wrap as directed on p. 78

Weigh the surplus material, which should, roughly, equal the theoretical excess—if the difference is large it may indicate inaccurate weighing and the exercise should be repeated

## EXAMPLE 6 1

Assuming 1 grain of Strychnine Hydrochloride is to be mixed with 63 grains of Lactose, the first addition of Lactose would be 1 grain, making  $1 + 1 = 2$  grains of mixture, the next addition would be 2 grains, making  $2 + 2 = 4$  grains of mixture, then progressively, 4, 8, 16 and 32 grains are added. In practice, the full bulk of diluent is weighed out, and each addition estimated by eye. The term *trituration* or 'triturate' is applied to a dilution of a potent substance with an inert one made in this way.

Admixture should be effected on a sheet of paper, using a flexible spatula or by rubbing together in a glass mortar.

Powders lightly triturated in this manner are more readily miscible with water than those heavily rubbed in a wedgewood mortar.

## EXERCISE 6 1

## Recipe—

Phenacetini	gr iv
Caffeinae	gr 1

Fiat pulvis	Mitte tales decem	Signentur	Capiat unum dolore
urgente			

## Quantity of Material

This exercise illustrates Rule (i) given above, i.e. it is practicable to weigh out material for one powder more than required, viz.—

Phenacetin	44 gr
Caffeine	11 gr

(continued overleaf)

**Exercise 6.1 continued**

Powder some Phenacetin and weigh out 44 grains of the powdered material, powder some Caffeine and weigh out 11 grains of the powdered material, thus completing Stage 1, p 79 At Stage 2 mix the Caffeine with about one-quarter of the weighed quantity of Phenacetin, and then add the remainder in approximately two equal portions, mixing well after each addition Complete Stage 3, and reserve the powders for Exercise 6.9

**EXERCISE 6.2**

Prepare 12 powders each to contain—

Aspirin	5 gr
Citric Acid	1 gr
Calcium Carbonate	1½ gr

Label—The Powders Dissolve in water and take when necessary

Calculate for 13 powders—

Aspirin	65 gr
Citric Acid	6½ gr
Calcium Carbonate	19½ gr

Powder an excess of each ingredient if not already in fine powder and weigh out the required amounts Triturate the Citric Acid with the calcium Carbonate gradually and then incorporate the aspirin Weigh out 12 × 7 grain powders and double wrap as directed previously

**EXERCISE 6.3**

*Receipt*—

Hydrargyri Subchloridi	gr ½
Sodii Bicarbonatus	gr ⅓
Rhei Pulverati	gr ss

Fiat pulvis Mitte tales duodecim Signentur Unus ter in die inter cibos sumendus

*Quantity of Material*

This exercise illustrates Rule (ii) given on p 79 One extra powder (13) would require a total of  $2\frac{3}{4}$  grains of mercurous chloride and thus quantity is not directly weighable It is, therefore necessary to prepare 15 powders

This exercise also illustrates Rule (iii)—the prescribed quantities totalling  $3\frac{7}{8}$  grains for each powder It will therefore, be necessary to adjust each powder to 4 grains by the addition of  $\frac{1}{8}$  grain of lactose

The formula for 15 powders will be—

Mercurous Chloride	3 gr
Sodium Bicarbonate	45 gr
Powdered Rhubarb	7½ gr
Lactose	4½ gr

All the above occur in fine powder, hence Stage 1 is superfluous and preparation begins with Stage 2, p 79 Mix the Mercurous Chloride and Lactose, add the Rhubarb and, finally, the Sodium Bicarbonate—adding one-third initially, and then the remainder Then complete Stage 3, and reserve the powders for Exercise 6.8

## EXERCISE 64

## Recipe—

Sodu Bicarbonatis	gr x
Rhei Pulverati	gr iii
Olei Menthae Piperitæ	m ss

Fiat pulvis Mitte novem. Signetur Unus e cyatho vino aquæ post cibos quoties opus sit capiendus

## Quantity of Material

This exercise illustrates Rule (iv) given on p. 79, i.e. it contains a fluid volume the weight of which could be found by calculation, but it would not be directly weighable. The prescribed amounts yield a powder weighing between 13 and 14 grains, hence adjustment to 14 grains with lactose will be suitable.

The formula for 10 powders will be—

Sodium Bicarbonate	100 gr
Powdered Rhubarb	30 gr
Oil of Peppermint	5 minims
Lactose	to 140 gr

The dry ingredients occur in fine powder therefore proceed directly to Stage 2, p. 79. The correct amounts of the first three ingredients are known but that of lactose is not. Therefore, after admixture of the first three ingredients, transfer the whole of the material to a scale, add lactose to produce 140 grains, return to the glass mortar, and mix thoroughly. Then proceed with Stage 3, wrap double because of the volatile oil, and reserve the powders for Exercise 6 10.

## POWDERS CONTAINING SMALL DOSES

As mentioned under Mixtures (p. 49) many substances are administered in doses which are a fraction of a grain. In some cases the prescribed dose is too small to be directly weighable but even when it is weighable (e.g. 1 grain), it is undesirable to send such small powders.

The following rules are suitable when the substance is ordered alone—

## For Imperial Quantities

*Make the weight of ingredient or triturate for each powder up to 2 grains with lactose*

## For Metric Quantities

*Make the weight of ingredient or triturate for each powder up to 100 mg with lactose*

Lactose is used as the diluent because it is colourless, soluble, and harmless—it is preferable to sucrose, which tends to absorb moisture, and cake.

## EXERCISE 65

## Recipe—

Codeine Phos	gr $\frac{1}{6}$
--------------	------------------

Fiat pulvis Mitte tales decem Signa Unus omni nocte sumendum

## Quantity of Material

This exercise not only illustrates the application of the above rule for small quantities in the Imperial system, but also exemplifies Rule (ii)—12 powders being made to obtain a directly weighable amount of Codeine Phosphate. Thus—

Codeine Phosphate	2 gr
Lactose (2 gr $\times$ 12) — 2 gr	= 22 gr

Both are fine powders, therefore proceed directly to Stage 2, p. 79, adding the Lactose in progressively increasing portions, and then completing Stage 3.

Example 6.2 gives the calculations necessary when before preparing the powders In practice, substances with very small doses are rarely prescribed the total quantity of ingredient cannot be weighed directly, i.e. when it is necessary to make a triturate in powders

## EXAMPLE 6.2

## Recipe—

Hyoscine Hydrobromide . gr  $\frac{1}{10}$   
Fiat pulvis Mitte duodecum. Sigma Capiat unum bis in die  
Quantity of Material Calculation for material for 15 powders will be  
convenient,  $15 \times \frac{1}{10} = \frac{1}{2}$  grain.

Triturate The following rule should be applied—

Use the nearest half-grain of ingredient above the amount required, with a minimum of one grain, and add to it sufficient lactose to produce the same number of grains of mixture as the figure in the denominator of the fraction required

Applying this rule, 1 grain of Hyoscine Hydrobromide must be mixed with sufficient lactose to yield 10 grains, i.e. 9 grains of lactose must be added, in the manner directed. This product will contain—

1 grain in 10 grains, i.e.  $\frac{1}{10}$  grain in 1 grain

The powders are now made, applying the rule for small quantities in the Imperial system, as follows—

Triturate of Hyoscine Hydrobromide (as above)	1 gr
Lactose (2gr $\times$ 15) — 1	= 29 gr

Stage 2 would then be completed, followed by Stage 3, forming 12 powders, each of 2 grains

*Alternative Method* The following is as satisfactory but more wasteful—  
1 powder must contain  $\frac{1}{10}$  gr made up to 2 gr with lactose

150 powders must contain  $\frac{1}{10} \times 150 = 15$  gr, made up to  $150 \times 2$  gr with lactose = 300 gr

The formula is thus—

Hyoscine Hydrobromide	1 gr
Lactose	299 gr

Stage 2 would then be completed, and Stage 3 by weighing and wrapping 12 powders each of 2 grains

## EXERCISE 6.6

Prepare 12 powders, each containing—

Aspirin	4 gr
Phaeacetin	4 gr
Codeine Phosphate	$\frac{1}{8}$ gr

Calculate for 13 powders—

Aspirin	52 gr
Phenacetin	52 gr
Codeine Phosphate	$1\frac{1}{8}$ gr

To obtain  $1\frac{1}{8}$  grains of Codeine Phosphate weigh—

2 grains ( $\frac{16}{8}$ ) + 14 grains of lactose, i.e. 2 gr in 16 gr  
 $\frac{1}{8}$  grain in 1 grain  
 $1\frac{1}{8}$  grains ( $\frac{17}{8}$ ) in 13 grains

Prepare the trituration of Codeine Phosphate in the manner described on p 79 and complete the incorporation of the other powders. Weigh twelve 9-grain powders and check the weight of the remaining powder. Pack and label

## Class 2. Powders Enclosed in Cachets and Capsules

Enclosure in cachets and capsules provides a means of administering nauseous or disagreeable powders in a tasteless form

### CACHETS

(Also called Wafer Capsules Latin *Oblatum*, or *Capsula Amylacea*)

The *cachet* is an improvement upon the wafer sheet at one time used to enclose nauseous powders before administration. Cachets are made of wafer sheet—a material made by pouring a mixture of flour and water between two, hot, polished, revolving cylinders. The water is thereby evaporated and a sheet of wafer formed.

There are two kinds of cachets, one called 'wet seal', because water is used to seal them, and the other 'dry seal'. Both kinds are made in a wide range, to contain from 1 to upwards of 20 grains

#### Wet seal Cachets

These consist of two halves, both alike, convex in shape, and provided with a broad flange. The powder is deposited in one half, the margin of the other is moistened with water, placed exactly over the filled half and the flanges pressed together

#### Cachet Machine for Wet-seal Cachets

These are made in several types, and are convenient for rapid work

The machine consists of three thin metal plates joined by hinges, enabling them to be opened or superposed. Each plate is pierced with two or more sets of similar holes, so that the machine may be used for two or more sizes of cachets.

With the plates laid open, one half of the cachet is placed in a hole in the centre plate, where it fits loosely. One of the side plates (the weaker one when there is a difference) is superposed on the central plate, thus covering the margin of the cachet and preventing powder from falling thereon.

The cachet funnel is then placed in position, and the powder pressed down into the cachet with a special thumble. The other half of the cachet is then placed in the corresponding hole of the third plate, where it is securely held. The covering plate is then turned back, the flange of the empty half of the cachet moistened with water, and the plate turned over into position so that the two halves of the cachet are joined by slight pressure. The cachet is then removed, by pressing upwards from below, and

allowed to dry. Needless to say, the full number of cachets are filled before any are sealed.

A perfectly sealed cachet without curled edges is not obtained unless there is just the correct dampness on the flange. The moisture is applied by means of a felt-covered roller, which should be wet but not excessively so. It is rolled carefully over the cachets to wet thoroughly the flanges. As the flanges may not be perfectly flat, it will be found satisfactory to roll the instrument along the line of cachets and back again, followed by two similar movements at right angles. The moistening process should take only two or three seconds.

After drying for about 15 minutes the cachets should be tested by gently pressing the edges by rotating between thumb and forefinger.

If no machine is available, two wide-mouthed bottles may be used, provided the flange just rests on the mouth of the bottle. The cachet is filled resting on one bottle, the other half moistened at the flange and superposed, and the cachet then covered with the second bottle inverted, pressing the bottles together will seal the cachet.

#### Cachet Machine for Dry-seal Cachets

These cachets consist of two halves, the 'cap' being slightly larger. They resemble a pill box lid, with a central 'stud' to fix them in the machine. The latter comprises two hinged plates, pierced with holes the size of the stud on the cachet. With the plates open, the smaller half of the cachet is placed on the lower plate—the stud fitting loosely into one of the holes. A loose plate with holes about the size of the cachet is then placed in position, and serves to guide the powder into the cachet. The powder is then poured through the hole of the loose plate, which is then removed. The cap of the cachet is pressed into the corresponding hole of the second plate of the machine—its stud fits tightly so that the cachet cannot fall when the plate is folded over. The plate is then well pressed down, forcing the cap over the lower half.

When substances likely to interact are prescribed together in a cachet, it is necessary to use *Bi* *cachets*. Both halves are filled, the partition fixed on one half, and this is subsequently moistened and pressed on to the other half.

#### Packing

Cachets are sent out on edge in cachet boxes, and the box, or compartments, filled with cotton wool when

necessary. The directions for use should read 'Hold the cachet by the edge, immerse in water until softened, and then swallow with the water.'

#### Recording

The size of the cachet used should be recorded in the practical book. In practice, it should be pencilled on the prescription.

#### CAPSULES

These consist of a body and a cap, made of gelatin. Although quite hard, they readily soften and dissolve upon swallowing with water.

The apparatus for filling them with powder is as follows—

- A block of wood, with holes of a suitable size
- A set of aluminium funnels narrowed to fit the capsules
- A thin glass rod or stick to act as a plunger

The bodies of the capsules are placed in the holes and the powder pressed through the funnel by the

plunger, and the margin of the body moistened with a camel hair brush before the cap is pressed on. This effects a perfect seal between body and cap.

In addition to powders, hard gelatin capsules are used to enclose ichthammol, phenol, and similar substances. They are made into a pill mass (see p. 94), then into pills, which are rolled to form cylinders. These are placed in capsules and sealed.

#### Packing

Capsules are sent out in pill boxes with directions to take them with water as they may cause temporary pain during solution in the stomach.

Weigh out powders, each of 200 mg and fill into capsules.

#### Enteric Capsules

As mentioned on p. 95, it is sometimes desirable to give medicaments in such a form that they will not be liberated in the stomach (Exercise 6.15).

#### EXERCISE 6.7

##### *Recipe—*

Carbo

gr x

Fiat capsula amyacea Mitte xii. Signa Una post cibos sumenda

Wet seal cachets should be used. The finished cachets, when held to the light, will at once indicate whether filling and sealing has been carried out successfully. Clumsy filling will be shown up by black specks between the flanges, and any gap in the sealing will be readily apparent.

#### EXERCISE 6.8

Transfer the Powders prepared for Exercise 6.3 to wet-seal cachets.

#### EXERCISE 6.9

Transfer the Powders prepared for Exercise 6.1 to dry-seal cachets.

#### EXERCISE 6.10

Transfer the Powders prepared for Exercise 6.4 to dry seal cachets.

#### EXERCISE 6.11

##### *Recipe—*

Hyoscinae Hydrobromidi

0.6 mg

Fiat capsula Mitte tales x Signa—Una quaque quarta hora

##### *Calculation*

6 mg will be required for ten doses. Each should be made up to a satisfactory weight with lactose. Therefore, make 100-mg powders. Prepare the following triturate—

Hyoscine Hydrobromide

50 mg

Lactose (to 500 mg)

450 mg

It will be convenient to prepare for 15 powders. This will give an excess and more convenient quantities than 11 powders.

Triturate                            90 mg (= 9 mg Hyoscine Hydrobromide)  
 Lactose (to 1,500 mg) 1 G 410 mg  
 Mix thoroughly and weigh out 10 powders and fill into capsules. Weigh the remainder as a check

**EXERCISE 6.12***Recipe—*

Quinalbarbitoni Sodii                            100 mg  
 Fiat capsula   Mitte tales v   Signatur—Una nocte sumenda

**EXERCISE 6.13**

Cyclizine Hydrochloride 25 mg

Prepare six capsules

Label—Travel Sickness Capsules One to be taken before going on board and one daily during the voyage

Prepare for seven capsules—

Cyclizing Hydrochloride	175 mg
Lactose, to 700 mg	= 525 mg

Mix as previously directed and weigh out six 100 mg powders and check the weight of the remaining powder Fill the powders into six capsules, pack and label

**EXERCISE 6.14***Recipe—*

Digitalis Präparata                            200 mg  
 Fiat capsula   Mitte tales duodecim   Signatur   Una ter die sumenda

**EXERCISE 6.15**

Prepare twelve enteric coated capsules each containing 100 mg of Emetine and Bismuth Iodide

Label—One to be taken every day

Prepare the capsules in the usual way and apply three coats of cellulose acetate phthalate using a 10 per cent solution in acetone (see p 95) Place each capsule in the solution, remove it with tweezers and touch off the excess fluid Place on a muslin strainer to dry Repeat with the other capsules Apply two further coats in the same manner

To test the efficiency of the enteric coating carry out the test for the disintegration of enteric-coated tablets described on p 1158 of the Pharmacopœia,

**Class 3 Moulded Tablets or Tablet Triturates**

These may be regarded as powders moulded to form a tablet They are quite different from compressed tablets (p 98), and are prepared extemporaneously with a small dose of some remedial agent, diluted with lactose They retain their shape under normal conditions, and may be swallowed whole or crushed to powder

The apparatus used is made of vulcanite or stainless steel, and consists of an upper plate with perforations corresponding in size, position, and number, with a range of pegs fixed in a lower plate, two large pegs ensure correct fitting of the plates

The medicated lactose is made into a stiff paste with 60 per cent alcohol, and then pressed into the perforations, a spatula is used to ensure that each cavity is filled, and to smooth off excess The filled plate is then superposed and pressed down, thus leaving the paste in the form of a tablet, resting on the pegs The tablets are left there to dry for one or two hours, and then packed into pill-boxes

Plates are made in a range of sizes,  $\frac{1}{2}$ -4 grain, and will prepare 50-250 tablets at one time Some plates are designed to produce tablets in triangular or other shape

**Calibration of the Mould**

The exact capacity of each mould must be determined before use. It may then be marked on the back of the mould to save further labour. Calibration is effected thus—

Place about 120 grains of lactose in a mortar, make into a stiff paste with 60 per cent alcohol, fill the perforations and press out the tablets. Dry them for at least 24 hours, to ensure complete volatilisation of the alcohol. Weigh only those tablets which are perfect, calculate the weight for the whole number, and record.

**Displacement Value of Medicaments**

When the proportion of medicament in a tablet triturate is small, any difference between its density and that of lactose may be ignored. However, when the proportion is appreciable, and its density differs considerably from that of lactose, a suitable correction must be made in order to ensure the correct content of medicament. In some instances it is necessary to find the proportion of medicament which displaces one part of lactose (called the displacement value).

Example 6.3 shows the method of finding the displacement value.

**EXAMPLE 6.3**

To find the displacement value of Calomel

Prepare an admixture of calomel 20 per cent and lactose 80 per cent, make into tablets, dry for 24 hours, weigh, and from the data obtained find the correct 'lactose displacement value' of the calomel. The following is an example of the calculation—

10 tablets containing 20 per cent of calomel and 80 per cent of lactose weighed 1.215 G. The composition of the 10 tablets is, therefore—

$$\begin{array}{l} 0.243 \text{ G calomel, and} \\ 0.972 \text{ G lactose} \end{array}$$

Now 50 tablets containing lactose only, weight 80 gr = 5.184 G,

10 tablets containing lactose only = 1.037 G

The calomel has, therefore, displaced 1.037 - 0.972 = 0.065 G of lactose

The displacement value of calomel is, therefore—

$$\frac{0.243}{0.065} = 3.74$$

**EXERCISE 6.16***Recipe—*

Strychninæ Hydrochloridi gr.  $\frac{1}{4}$

Fiat tabletta Mitte duodequinquaginta Signa Una post cibos sumenda

*Calculation*

The tablets should weigh about  $\frac{1}{2}$  grains. Assume that the mould held 50 tablets, weighing 80 grains. For the present exercise it is convenient to make for 60 tablets to allow for loss, and for convenience in weighing, and, consequently, 96 grains of triturate will be required.

The formula will be—

Strychnine Hydrochloride	1 gr
Lactose (96 - 1 gram)	$= 95 \text{ gr}$

Proceed as indicated above

**EXERCISE 6.17**

Prepare 21 tablet triturates, each containing 2 milligrammes of Calomel  
Label One to be taken three times a day

*Calculation*

Calomel is a dense substance, and if the tablets are made in a mould of similar size to that used in Exercise 6.16, they will contain about 20 per cent

of calomel! Hence a correction must be made for the displacement value of the calomel, and the figure given in the example on p. 86 may be used.

It will be convenient to prepare 25 tablets to cover loss, and, assuming that the same mould is used as in Exercise 6.16, 25 tablets of plain lactose will weigh 40 grains = 2 592 G

The formula will therefore be—

Calomel

0 500 G

This will displace

$$\frac{0.500}{3.74} = 0.133 \text{ of lactose}$$

$$\text{lactose required is } 2\ 592 - 0\ 133 = 2\ 459 \text{ G}$$

Mix, and proceed as before, covering half the upper plate with a powder paper to prevent unintentional distribution of the mass into too many of the perforations

#### Class 4 Powders in Granular Form, or Effervescent Preparations

These consist of a mixture of citric and tartaric acids with bicarbonate of soda, and usually some medicament, and occasionally sugar.

The following notes explain why these ingredients are present, and also indicate the method of preparation and the importance of the calculations required in certain instances.

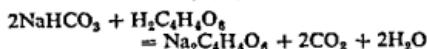
##### 1 Sodium Bicarbonate

This reacts with the acids when the effervescent preparation is added to water, the carbon dioxide evolved providing the effervescence.

##### 2 Citric and Tartaric Acids

The quantity of these is slightly more than is needed to neutralise the sodium bicarbonate because effervescent preparations are pleasanter when slightly acid.

The relative proportions of citric and tartaric acids is based on experience of the quantity of water needed to render the mixed material, in powder, coherent, ready for division into granules by forcing through a sieve. Tartaric acid is anhydrous but citric acid contains 1 molecule of water of crystallisation, equal to 8.75 per cent of its weight. Heating for granulation liberates this water, and the moist condition thus produced allows partial interaction between the acids and the sodium bicarbonate, so that more water is formed.



The water from these two sources, i.e. the water of crystallisation of the citric acid and that formed by

reaction between the acids and the bicarbonate, renders the material coherent.

The citric acid therefore serves two purposes—it provides most of the moisture needed for granulation (and thus provides conditions for the release of more moisture), and neutralises part of the sodium bicarbonate. The tartaric acid provides the remainder of the acid required for slight over-neutralisation of the sodium bicarbonate.

It should be noticed that loss of weight occurs in granulation, from two factors—

(a) Loss of moisture by evaporation from the damp mixture

(b) Loss of carbon dioxide in the above reactions

These losses together amount to approximately one-seventh of the weight of powder used, and they must consequently be allowed for in calculating for medicated effervescent preparations. Examples of the calculations involved are provided in the exercises which follow.

##### 3 Medicament

In many cases the medicament is a crystalline inorganic salt containing water of crystallisation, e.g. magnesium sulphate, sodium sulphate. In other cases the medicament is a substance not containing water of crystallisation. The medicament must be freed from any water of crystallization which is liberated at or below 100°C, which is the temperature reached in the granulating process. Care must be taken not to confuse this with excitation. Assume for the moment that the medicament is a salt containing 7 molecules of water of crystallisation which are lost as follows—

$$3\text{H}_2\text{O lost at } 60^\circ\text{C}$$

i.e. this water would be liberated from the hydrate with  $7\text{H}_2\text{O}$  at the temperature of granulation, to form the hydrate with  $4\text{H}_2\text{O}$

$4\text{H}_2\text{O}$  lost at 160°C

i.e. this water would be absorbed by the exsiccated salt at the temperature of granulation to form the hydrate with  $4\text{H}_2\text{O}$

It is evident therefore in this example that—

- Failure to remove water of crystallisation liberated at or below 100°C will lead to liberation during granulation and the water thus liberated will enable the acids to react completely with the bicarbonate and the finished product would not therefore effervesce
- Removal of all the water of crystallisation, i.e. exsiccation, would lead to absorption of the water released from the citric acid (and needed for granulation), and therefore granulation could not be effected.

#### 4 Sugar

This is included in some effervescent preparations as a sweetening agent

#### METHOD FOR EFFERVESCENT PREPARATIONS

##### STAGE 1

Place a porcelain \*dish on a water bath with the dish deep in the bath. Heat the bath to boiling-point, and maintain for Stage 3

A hot dish over a boiling water bath ensures rapid liberation of the water of crystallisation from the citric acid, and assists in producing a coherent mass. If heating is not started until the powder is placed in the dish, the water of crystallisation is released slowly as the temperature rises, and a high proportion

\* An enamelled iron dish is a poor conductor of heat and is not suitable

of it is lost by evaporation at approximately the same rate as liberation. Consequently the optimum proportion of moisture needed to render the powder coherent is not present at any time, and failure ensues

##### STAGE 2

Finely powder all the ingredients, any salt which has been exsiccated must be reduced to fine powder before being mixed with the other ingredients. When sugar is required, powdered (icing) sugar must be used. Thoroughly mix all the powders in ascending order of weight

##### STAGE 3

Place all the powders in the hot porcelain dish on a boiling water-bath

##### STAGE 4

Press down with a bone spatula or scoop until the mixture has formed either a loose cake or a damp coherent mass. If the former, reverse the cake to heat the upper surface. This operation usually takes about 1-5 minutes

##### STAGE 5

Have ready a No. 8 sieve superposed upon a No. 20 or 24, and quickly press the mixture through the top sieve and then shake. The finest granules will drop through the lower sieve, leaving upon it practically uniform granules which constitute the required product

##### STAGE 6

If time permits, spread the granules out on a tray in a warm place, and dry for 2 or 3 hours

##### Packing

Dry granules are sent in bulk in a wide mouthed bottle, or separately wrapped in double powder papers (p. 78), and labelled as instructed

#### EXERCISE 6 18

Prepare 40 G of Sodium Citro-tartrate Effervescent Granules, B.P.C.  
1959  
Label The Effervescent Granules  
Follow the Method for Effervescent Preparations

#### EXERCISE 6 19

Prepare 30 G of Effervescent Citro-tartrate of Soda containing 5 per cent of Iron and Ammonium Citrate  
Label One teaspoonful to be taken in water after each meal.

##### Calculation—

In the finished preparation there are 95 parts of Effervescent Citro-tartrate of Soda with 5 parts of Iron and Ammonium Citrate

The B P C quantities for Effervescent Citro-tartrate of Soda produce approximately 1,000 G of granular matter, and one twenty-fifth will therefore produce 40 G of granular base

The quantity of Iron and Ammonium Citrate which must be added to this is

$$\frac{40 \times 5}{95} = 215 \text{ G}$$

The formula for making is therefore—

Sodium Bicarbonate, in powder	20 40 G
Tartaric Acid, in powder	10 80 G
Citric Acid, in powder	7 20 G
Refined Sugar, in powder	6 00 G
Iron and Ammonium Citrate, in powder	2 15 G

Mix in ascending order of weight, granulate as directed on p 88 Finally, from the uniform granules, weigh out 30 G, and complete as stated on p 88

#### Class 5 Powders for External Use

##### Forms of Powders for External Use

These comprise—

(a) Dusting Powders (Latin *Pulvis Conspersus*)

Dusting Powders are powders in a fine state of subdivision for external application. They are usually mixtures of such substances as zinc oxide, starch and boric acid or natural mineral substances such as kaolin or talc. The latter may be contaminated with pathogenic organisms such as *Clostridium tetani* etc and should, therefore, be sterilized by dry heat (Chap 22). Dusting powders are not intended for, and should not be used for application to areas where the skin is broken. They are sent out in dredgers

(b) Insufflations (Latin *Insufflatio*)

These are really dusting powders which are intended to be blown by means of an insufflator, in order to reach areas to which the powder could not be applied directly

Insufflations are usually sent out in wide-mouthed

bottles, from which small quantities are transferred to the insufflator as required

(c) Snuffs (Latin *Sternutamentum*)

These are sent out in flat metal boxes with a hinged lid, a shallow pill-box would answer, provided the ingredients were not volatile

#### METHOD FOR POWDERS FOR EXTERNAL USE

##### STAGE 1

Separately powder a slight excess of each crystalline ingredient

##### STAGE 2

Weigh out the required quantities of each ingredient, and mix them gradually in ascending order of weight, as described on p 79

##### STAGE 3

Pass the material through a No 80 sieve

#### EXERCISE 6.20

##### Recipe—

Zinci Oxidi	20 0
Acidi Salicylici	2 0
Amyli Pulverisat;	78 0

Fiat pulvis subtilis Mitte 3 j Signa Pulvis conspersus

Prepare 500 grains for convenience of calculation and to cover loss  
Follow the method described above

#### EXERCISE 6.21

Boric Acid }  
Zinc Oxide } of each  $\frac{1}{2}$  oz.

Label The Dusting Powder To be applied to the face and neck frequently

### POWDERS CONTAINING LIQUIDS OR LIQUEFIABLE SUBSTANCES

Certain substances tend to liquefy when mixed together, e.g. an oily liquid is produced when any two of the following are mixed—menthol, phenol, and camphor. Powders containing two or more of these substances are dealt with as in Exercises 6.22 and 6.23.

**Method for Powders containing Liquefiable Substances**

#### STAGE 1

Separately powder a slight excess of each crystalline ingredient

#### STAGE 2

Weigh out the required amounts of each ingredient and separately and gradually dilute each liquefiable substance with a portion of the other ingredients, then mix these portions

#### STAGE 3

Pass the material through a No. 80 sieve

#### Iodine in Dusting Powders

As already seen, the use of a solvent facilitates subdivision and distribution throughout the other ingredients, and this fact is utilized in preparing powders containing iodine (and also certain resinous substances). It is, of course, essential to select a readily volatile solvent. Exercise 6.24 illustrates the method

### EXERCISE 6.22

Prepare 25 G of Dusting Powder of Zinc Undecenoate B.P.C.  
Label The Dusting Powder  
Follow the method of the Codex

### EXERCISE 6.23

*Recipe—*

Menthols	5
Camphoræ	5
Ammonii Chloridi	30
Magnesi Carbonatis Ponderosi	60

Fiat insufflatio Mitte 3 iv Signetur Pro naso  
Follow the *Method for Powders Containing Liquids or Liquefiable Substances*, described above

### EXERCISE 6.24

Send  $\frac{1}{2}$  oz of Boric Acid containing 1 per cent of Iodine

Iodine is soluble in alcohol, and this will serve as a distributive agent. The Pharmacopœia includes an alcoholic solution of iodine, Simple Solution of Iodine, which contains 9 per cent w/v of iodine. It will be convenient to prepare 300 grains of the powder, using 37 minims of the official solution and 297 grains of powdered boric acid. If the solution is not available, 3 grains of powdered iodine may be dissolved in 30 minims of 95 per cent alcohol\* or 40 minims of 90 per cent alcohol\*. Iodine is very volatile, hence the alcohol must be allowed to evaporate at room temperature. Further, iodine reacts with metals, and a hair sieve must, therefore, be used. This preparation should be dispensed in a bottle.

\* Industrial Methylated Spirit should not be used.

### STANDARDISED SIMPLE POWDERS

The B.P. and B.P.C. lists 5 drugs in powder form, each adjusted to a defined strength. The general method of preparation is as follows—

The drug is reduced to fine powder, and a portion subjected to analysis to determine the proportion of

active constituent present. The remainder is then adjusted to the defined standard in one or more of the following ways—

- (a) By admixture with a suitable proportion of powdered drug of higher or lower potency

- (b) By dilution with the corresponding exhausted drug remaining after a process of extraction  
 (c) By dilution with lactose or other diluent

Powdered lucerne and grass are specified as suitable diluents for Prepared Digitalis

Powdered cocoa husk or lactose coloured with burnt sugar are specified as suitable diluents for Powdered Opium

The official standardised simple powders are set out in Table 6 1

The standardised simple powders provide—

1 Drug, in fine powder, and of constant composition, for use in dispensing pills, powders, capsules, cachets, and similar solid or semi solid preparations

2 Material, which can in emergency replace any other oral preparation of the drug

3 Material which is stable, the powdered drug being, in some cases, the most stable form in which the drug can be exhibited For example, Tincture of Digitalis, now without B P or B P C recognition loses potency slowly, but the loss is appreciable over

a period of about a year Powdered Digitalis, if kept quite dry, remains fully potent for years—a fact attested by the selection of *Digitalis* in powder form as the International Standard for Digitalis

4 Material which is portable with safety as compared with fluid preparations

5 Material which provides a concentration of active principle which, in most cases, is as high or higher than that represented by fluid (and therefore less safely portable) preparations of the drug A dry extract does, of course, provide a high concentration of active principles, but only one, Belladonna, of the drugs official as standardised powder are also official as dry extracts, in the other cases the standardised powder is the most concentrated solid form available.

6 Inexpensive material Preparation of extracts of a drug demands the use of solvents, labour costs for processing, and apparatus, as well as the two processes involved in preparing standardised powders, i.e. comminution of the drug, and assay Standardised powders can therefore be produced more cheaply than the equivalent weight of an extract

*Table 6 I*  
Official Standardised Simple Powders

Name	Method of adjustment	Official Standard
Prepared Belladonna Herb	(a) or (b)	0.3% belladonna alkaloids
Prepared Digitalis	(a) or (c)	10 units per G
*Prepared Ergot B P C	(a)	0.15% ergot alkaloids
Prepared Ipecacuanha	(b) or (c)	2.0% ipecacuanha alkaloids
Powdered Opium	(c)	10% anhydrous morphine

\* The ergot is defatted by percolation with light petroleum and then dried at a temperature not exceeding 40°C before assay and adjustment. If Method (a) is used for adjustment the added drug is similarly defatted

### B.P.C. COMPOUND POWDERS

These are prepared as described on p 79 (*Method for Compound Powders*) Many of the ingredients are available in a finely-powdered condition—some of them could not be reduced to fine powder without mechanical means

#### Powder of Bismuth, Compound

Bismuth Carbonate

Calcium Carbonate

Heavy Magnesium Carbonate

Sodium Bicarbonate

#### Powder of Chalk, Aromatic

Chalk

Cinnamon

Nutmeg

Clove

Cardamom

Sucrose

#### Powder of Chalk with Opium, Aromatic

Aromatic Powder of Chalk

Powdered Opium

This powder contains 0.25 per cent of anhydrous morphine constituent of the Seidlitz spring in N.E. Czechoslovakia

**Compound Effervescent Powder (S) n Seidlitz Powder**

Sodium Potassium Tartrate  
Sodium Bicarbonate

These are wrapped in blue paper

**Tartaric Acid**

This is wrapped in white paper

The powder is administered as an effervescent draught, the contents of the blue paper being dissolved in half a tumblerful of water and the contents of the white paper added, the product being drunk immediately.

As mentioned under Effervescent Mixtures (p. 87) preparations of this kind are more agreeable when slightly acid, and the salty taste of the ingredients is then less noticeable. Seidlitz Powder contains 12 per cent more tartaric acid than necessary for neutralising the sodium bicarbonate. The draught contains sodium potassium tartrate together with the sodium tartrate formed by interaction of the tartaric acid and the sodium bicarbonate. Hence when it is desirable to increase the strength the sodium potassium tartrate in No. 1 powder is augmented. Extra Strong Seidlitz Powder B.P.C. contains half as much again of sodium potassium tartrate while Double Strength Powders should contain twice the normal quantity.

The Seidlitz Powder was introduced in 1815 by T.F. Savory to represent the principal aperient

Powder of Kaolin, Compound

Light Kaolin

Heavy Magnesium Carbonate

Sodium Bicarbonate

Powder of Liquorice, Compound

Senna Leaf

Liquorice peeled

Fennel

Sublimed Sulphur

Sucrose

Powder of Magnesium Carbonate, Compound

Heavy Magnesium Carbonate

Calcium Carbonate

Sodium Bicarbonate

Light Kaolin

Powder of Magnesium Trisilicate, Compound

Magnesium Trisilicate

Heavy Magnesium Carbonate

Chalk, in powder

Sodium Bicarbonate

Powder of Rhubarb Compound (S) n Gregory's Powder)

Rhubarb

Heavy Magnesium Carbonate

Light Magnesium Carbonate

Ginger

#### REVISION EXERCISES 1 Recipe—

Rhei	partes xxx
Zingiberis	partes xii
Hydrargyr Subchlorida	partes iv

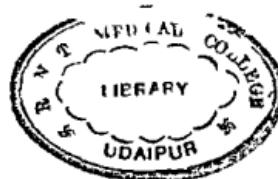
Sit dosis grana decem. Mitte tales sex in oblatis S gna Unum omni mane ante jentaculum sumendum.

#### 2 Recipe—

Audi Salicylici	5
Amyli	20
Zinci Oxidi	20
Crete Gallica	40

Fiat pulvis conspersus. Mitte  $\frac{3}{4}$  l.

- 3 Prepare two Extra Strong Seidlitz Powders B.P.C.
- 4 Prepare 1 oz (troy) of Effervescent Citro-Tartrate of Soda, containing 5 per cent of Phenazon® and 1.5 per cent of Caffeine Citrate. Label. Two teaspoonfuls to be taken in water, while effervescent, when headache starts.
- 5 Prepare 1 oz of Compound Powder of Rhubarb



## Pills

**PILLS** are small spherical or ovoid masses which were formerly commonly required to be made at the dispensing counter. Extemporaneously prepared pills are today a rarity. No formulae for pills are given in the *British Pharmacopœia*, and in the British Pharmaceutical Codex 1959 only four are included. Those formulae that remain are well known combinations for which a demand still exists and in these cases the manufactured article is usually available.

Extensive details for making pills of different kinds were given in the previous edition of this book but the following descriptions should be adequate for present day needs.

### 1 'Solubility'

Ready disintegration in the intestinal tract is naturally an all important requirement. Most pills when freshly made fulfil this condition. In large scale manufacture, pills are usually coated with sugar or talc (pearl coating). If these coatings are applied to the pills when freshly made and plastic, the pill-mass slowly dries and shrinks within the coating, which cracks and breaks off during transit. This is usually prevented by a process called 'ripening', i.e. exposing the uncoated pills in shallow trays to a hot atmosphere until quite dry and hard. Pills produced in this manner are less soluble than freshly made pills, and may sometimes pass through the intestinal tract without disintegrating.

### 2 Homogeneity

If the dosage is to be accurate it is essential that the medicament should be thoroughly and evenly distributed throughout the pill mass. Uncoated pills should satisfy the requirements of the *British Pharmacopœia* for Uniformity of Weight of Tablets (see p 109).

### 3 Uniformity in Weight

This must be coupled with homogeneity, in order to ensure accurate dosage.

### 4 Suitability of Shape

To facilitate swallowing, pills should be round or oval. In theory the oval pill has an advantage, because the shorter diameter facilitates entry into the œsophagus.

Pills should not be too large or too small for convenience in handling and swallowing. The British Pharmaceutical Codex gives a general recommendation that pills should not be less than 3 mm in diameter for pills weighing up to 1 grain and not usually more than 8 mm for pills weighing about 5 grains.

### 5 Tastelessness and Elegance

For preference, pills should be coated. Sugar-coating or varnishing does not appreciably delay disintegration, because these coatings are quickly washed off in the intestinal tract. Coating not only renders pills tasteless and elegant, but also minimises changes in the composition of the pill.

## FORMULATION

Pills are made from pill masses, which may contain three classes of substances—

- 1 The medicinally active ingredients
- 2 A diluent—this is necessary when the quantity of active ingredients is very small
- 3 The excipient or substance required to form a mass. The latter must be firm, plastic, adhesive, and also soluble in the gastric fluid. The excipient may consist of—
  - (a) A binding agent, which assists adhesion of the particles when (c) is added,
  - (b) An absorbing agent, necessary in pills containing oily ingredients, and
  - (c) A fluid or semi solid to render the mass plastic

The correct functioning of the excipient is among the most important factors in successful pill making.

### Preparation of Pills

For description, this may be conveniently divided into—

- A Preparation of the pill mass**
- B Rolling cutting and rounding**
- C Coating, and**
- D Packing**

Much of the above can be learned only from demonstration and practice the following account is intended to supplement demonstration by the teacher

#### **A Preparation of the Pill Mass**

##### **STAGE 1**

Thoroughly mix all the dry ingredients in ascending order of quantity By dry ingredients is meant solid substances, whether medicinally active or part of the excipient, all must be in very fine powder Admixture is made in a pill mortar, which differs from an ordinary mortar in being shallower, having rounded edges, and no spout The pestle used with a pill mortar should be about 8 in. long almost cylindrical, and have a well rounded end, so that it will fit comfortably into the palm of the hand when kneading the pill mass

##### **STAGE 2**

Add any fluid ingredients, and distribute thoroughly by trituration.

##### **STAGE 3**

Add the fluid excipient, using at first rather less than the anticipated amount, and then adding a very little at a time until a proper mass is formed. During this operation the mass generally requires loosening from the sides of the mortar, and for this purpose a short non flexible spatula with a broad blade is most suitable In general, the mass is completed when it tends to peel from the sides of the mortar, it may then be removed from the mortar, and kneaded between the fingers

#### **B Rolling, Cutting, and Rounding**

The whole of the mass or the weighed portion required, is then rolled out on the flat board of the pill machine to the exact length for the number of pills required It is most important that the pill pipe, as it is called should be as perfectly cylindrical as judgement by eye can make it, otherwise the pills will be unequal in size

Rolling is usually started beneath the fingers and continued and completed beneath the back of the cutter or the bottom of the pill tray The cutters

are usually dusted with powder to prevent adhesion of the pills for this purpose, powdered liquorice root is used for dark pills, and kaolin for light

The pipe is then laid on the fixed cutter so that it fits the number of divisions corresponding to the number of pills into which it is to be cut. The upper cutter is pressed lightly on the pipe, a quick to-and fro movement started, and the pressure on the upper cutter gradually increased until the cutting edges meet The to-and fro movement of the cutter should be maintained for about a minute after the pipe has been cut through

As cut, the pills are not usually quite round. To finish them they are rapidly rotated beneath a boxwood pill rounder, a little powdered French chalk (talc) being dusted over the pills to make them rotate easily and smoothly The rounded pills are then placed on a pill rounder and gently rubbed with a powder paper to remove the talc as completely as possible This procedure is particularly necessary when the pills are to be coated with varnish or gelatin

#### **C Coating**

Coated pills are more elegant and often more stable than uncoated pills but a coating need not be applied unless specified.

Methods which have been used for coating pills include varnishing, sugar-coating, pearl-coating, gelatin-coating silvering and gilding These methods were described in the previous edition of this book but since the extemporaneous preparation of pills is now rare only varnishing is described as a general method Since, however, enteric-coated pills may be required in an emergency a simple, effective method of enteric coating is also described.

#### **Varnishing**

The varnish used is a sandarac varnish, made up from the following formula—

Sandarac	1 part
Absolute Alcohol	2 parts
Ether	2 parts

Dissolve in a closed bottle by occasional shaking Decant the clear liquid from any debris

#### **Method**

Very slightly smear a white porcelain slab, and the end of a glass rod, with almond oil Place one drop of varnish per pill into a flat-covered pot, add the pills cover and rotate quickly for about 15 seconds

Gently shake the pills on to the slab, and, after half a minute, turn each pill over. Repeat this after an interval of about 1 minute, and then leave to dry. In some cases the first coat of varnish is partially absorbed by the pills, causing them to appear dull, if this occurs the pills should be given a second coat.

#### Coatings for Enteric Pills

Enteric coating implies that the coating will withstand any disintegration effects in the stomach but will break up in the intestine. Such coatings are used for substances which irritate the mucous membrane of the stomach or are decomposed by stomach juices and also for substances such as anthelmintics and amoebicides which are intended for concentrated effect in the intestine.

Among the substances which have been used for the extemporaneous coating of pills, tablets and capsules are keratin, shellac, salol, stearic acid. Gelatin treated with formalin has also been used. These substances are somewhat difficult to apply elegantly and with the certainty of a true enteric effect. Their use is based on the fact that they are insoluble in hydrochloric acid and soluble in alkaline solution. The acidity of the stomach, however, varies considerably in individuals and at different times and, moreover, the intestine is sometimes acid in reaction. Newer methods of enteric coating, therefore, are independent of hydrogen ion concentration in stomach and intestines.

One fairly successful coating suggested by Stoklosa (1953) consists of a mixture of *n* butyl stearate, carnauba wax and stearic acid. This mixture has to be applied at about 75°C by a dipping process and it is not very easy to achieve products with an elegant appearance.

A more satisfactory method is described by Parrott (1961) involving the use of cellulose acetate phthalate solution in acetone. The capsule, pill or

tablet is dropped into a 10 per cent solution and removed with tweezers. The excess drop of fluid is touched off and the product drained on a muslin strainer. After drying, second and third coats are applied.

Cellulose acetate phthalate disintegrates readily in the intestine due to the ionic content (Malm 1951) and to the presence of hydrolytic esterases (Bauer 1948). Parrott recommends that disintegration tests should always be carried out in tablets coated in this manner because of the possibility of the tablet ingredients affecting the coating.

Several methods of enteric coating pills, capsules and tablets are trade secrets. In one proprietary brand of enteric coated tablets (Enseals) the disintegration depends on time and moisture and is, therefore, independent of the reaction of the stomach and intestine. This ensures success even in cases of achlorhydria and hypochlorhydria. The enteric coating is applied on a subcoat and consists of a mixture of oils, waxes and fatty acids of high melting point with moisture-absorbing fibres such as agar and powdered elm bark. A final sugar-coating is also applied. These tablets disintegrate in four to seven hours, the time being based upon the average emptying time of the stomach, which is three to five hours.

#### D Packing

Pills are sent out in shallow circular boxes of various kinds, the neatest being purple-shouldered pill boxes, with flanged edges. A disc of wax paper, or a thin circle of cotton wool, should be placed top and bottom, and the box should be large enough to take all the pills in one layer, to prevent them losing their shape. For wrapping the box, the paper should be cut so that the margin on each side is less than the depth of the box, the folded ends are turned upwards and sealed at the sides.

### PILL EXCIPIENTS

#### (a) Adhesive Excipients

- 1 Acacia, in powder form, is a powerful adhesive in the presence of moisture. From 5-10 per cent of the weight of pill mass is used—more than this proportion may cause the pills to harden excessively. It has a wide range of use with vegetable powders, in conjunction with Syrup of Liquid Glucose.
- 2 Tragacanth in powder, is used as an adhesive and for hardening soft masses. The usual proportion is about 5 per cent. A mixture of equal parts of

- 3 acacia and tragacanth is often superior to either Syrup of Liquid Glucose. This is less adhesive than liquid glucose, but it is more convenient. When used with acacia it forms a good general excipient.

#### (b) Absorbent Excipients

- 1 Kaolin
- 2 Liquorice Root, in powder, is an excellent absorbent, and, by virtue of its fibrous character, is ideal for stiffening soft masses.

- 3 Curd Soap, in powder, is much used for oils and  
only bodies, usually combined with liquorice root
- 4 Hard Soap, in powder, is used similarly to Curd  
Soap

**EXERCISE 7.1**

Prepare 24 Compound Pills of Phenolphthalein B P C  
Label as such

Each pill contains  $\frac{1}{6}$  of a grain of strychnine. Three tenths of a grain are, therefore, required for twenty-four pills. Weigh 1 grain carefully and gradually triturate it with 9 grams of lactose. Three grains of this trituration will contain  $\frac{1}{6}$  grain of strychnine. This should be thoroughly mixed with the other dry ingredients in ascending order of weight and the product massed with the Liquid Glucose Syrup in the manner previously described

**EXERCISE 7.2**

Prepare 30 Compound Pills of Colocynth B P C

Label as such

Note the use of Curd Soap to absorb the Clove Oil

**REFERENCES**

- BAUER, C W and MASUCCI, P E (1948) The Action of Intestinal Enzymes upon Cellulose Acetate Phthalate and Butyl Stearate Enteric Coated Tablets. *J Amer Pharm Ass (Sc Ed)* 37, 124-128  
'ENSEALS' ELI LILLY and CO., LTD  
MALM, C J, EMERSON, J and HIATT, G D (1951) Cellulose Acetate Phthalate as an Enteric Coating

Material, *J Amer Pharm Ass (Sc Ed)* 40, 520-525

PARROTT, F L (1961) An Extemporaneous Enteric Coating. *J Amer Pharm Ass (Pract Ed)* NSI 3, 158-159

STOCKLOSA, M J and OHMART, L M (1953) A Practical Method of Extemporaneous Enteric Coating. *J Amer Pharm Ass (Pract Ed)* 14, 507-515

## 8

## Compressed Tablets

THESE are small compressed masses containing a medicament or medicaments, officially circular in shape, and may be flat or bi convex. In most instances they contain additional substances necessary for their manufacture, disintegration, or appearance.

The manufacture of compressed tablets for medicinal use dates back to the first half of last century, when, in 1843, a Mr Brockdon took out a patent for 'Shaping pills, lozenges and black lead by pressure dies.' The general manufacture of compressed tablets, however, only assumed considerable proportions at the beginning of this century, when many new machines were devised and a considerable amount of investigation into methods of preparation was carried out.

Today, the use of medicinal tablets is very extensive, as they have largely replaced powders and cachets. In addition, many formulae, formerly dispensed as pills, are now dispensed in tablet form.

The following types of compressed tablets are in use for medicinal purposes—

- 1 Tablets which disintegrate readily when swallowed, e.g. Acetylsalicylic Acid Tablets. In such cases fairly rapid absorption is required.
- 2 'Lozenge' tablets. These do not disintegrate readily and are intended for slow solution in the mouth, usually to produce a local action on the throat.
- 3 Tablets to be dissolved in water for administration, either—
  - (a) orally, or
  - (b) by external application, or
  - (c) by parenteral injection

The term 'Solution-tablet' or 'Solvella' is used in the British Pharmaceutical Codex to designate a tablet which is to be dissolved in water to form a solution for external use.

- 4 Tablets which are to be chewed. These include Aluminium Hydroxide Tablets, in which mastication ensures thorough breaking up of the mass and diffusion of the antacid which acts mainly by adsorption. Also included in this group are Phenolphthalein Tablets, since this substance is absorbed mainly in the mouth.
- 5 Buccal tablets are to be placed in the buccal pouch where they dissolve or disintegrate slowly and are absorbed directly without passing into the alimentary tract. Certain steroids are presented in this way, e.g. Ethisterone Tablets.
- 6 Sublingual tablets to be placed under the tongue where they dissolve or disintegrate quickly and are absorbed directly without passing into the alimentary tract. Glyceryl Trinitrate Tablets are used in this way. The B.P. states that 'they dissolve slowly in the mouth.' The U.S.P. XVI gives a disintegration time of 2 minutes.
- 7 Implants. These may be made by heavy compression but are normally made by fusion. They are inserted subcutaneously by means of a minor surgical operation and are slowly absorbed. They are packed singly in sterile containers. Official examples are Deoxycortone Acetate Implants and Testosterone Implants.

The compression of the materials into tablets is carried out by means of a machine which stamps out the tablet in a die between punches. It must be appreciated, however, that the preparation of tablets requires a considerable amount of skill, a sound knowledge of materials and formulation, and a thorough acquaintance with the machine. It should

also be kept in mind that satisfactory tablets should have the following properties—

- 1 They should contain the correct dose
- 2 They should be satisfactory for their particular purpose, as outlined above
- 3 They should contain no unnecessary or excessive

adjuncts, and these should be as innocuous as possible

- 4 They should be capable of being handled and transported without crumbling
- 5 They should possess a good appearance with a smooth and uniform surface

### PREPARATION AND CHOICE OF MATERIALS

It is possible to make tablets, one by one, by compressing individually weighed quantities of powder between punches in a suitable die. The normal method, however, is to use a machine in which the material is fed automatically to the die. For various reasons the material must be in the form of *granules* and not in *fine powder*. These reasons are given in Table 81. A recently introduced device for powder feeding with rotary tablet machines has come into use in the U.S.A. So that its method of operation will be more readily understood this machine is described after the explanation of the rotary tablet machine on p. 107.

Some substances can be purchased in the form of small crystals, or as larger crystals which can be easily reduced to smaller size. The only preparation necessary in such cases is to crush the crystals, if too large, so that they just pass through a suitable sieve (No. 20 for average size tablets). They are then shaken over a finer sieve (No. 40) and any fine powder rejected. The granules are then ready for compression. The *British Pharmacopœia* describes this process as *dry granulation*.

In general, however, it is necessary to prepare the materials in the form of granules by the use of additional substances. These, and other necessary adjuncts, include the following—

- 1 Granulating agents
- 2 Binders
- 3 Lubricants
- 4 Disintegrating agents
- 5 Diluents, also called bases or fillers.

#### 1. Granulating Agents and Granulation

The methods of granulating are—

Dry granulation

Moist granulation

Granulation by preliminary compression

#### DRY GRANULATION

This method, described above, requires no granulating agent since the materials are always available in granular form.

Table 81

<i>Fine powder</i>	<i>Granules</i>
1 Fine powder does not 'flow' evenly through the hopper of the machine. The irregular supply of fine powder to the die produces tablets of varying weight	1 Granules flow' evenly and a given volume of granules is more constant than that of a powder
2 In a powder containing several ingredients the vibration of the machine would promote separation, the denser ingredients would tend to gravitate to the bottom of the powder and the tablets would not be uniform in composition	2 The granules are uniform in composition and the ingredients are bound together and cannot separate. Gravitation of the smaller granules to the bottom is, therefore, unimportant
3 Air is imprisoned during compression and may cause 'capping', i.e. splitting off of the upper surface of the tablet either as it leaves the die or subsequently (see also p. 118). Also the tablets tend to crumble during transport and handling	3 Upon compression the granules knit together to form sound tablets
4 Fine powder tends to blow out of the die at the top and to seep downwards round the stem of the lower punch, causing sticking	4 The granules being heavier do not blow out of the die and do not clog the lower punch

## MOIST GRANULATION

This is effected by the use of suitable fluid granulating agents. Those in common use are water, mucilages of acacia, tragacanth and starch, solutions in water of glucose, sucrose and gelatin, solutions in ether of cocoa butter and stearic acid, alcohol in various dilutions, isopropyl alcohol and acetone.

The powder is moistened with the granulating agent, using sufficient to render it coherent, so that it will 'ball' in the hand, but is not too damp. The coherent material is then passed through a suitable sieve. Any tendency of the mass to stick to the wire of the sieve indicates over-moistening. The sifted material, which is now in the form of granules, is dried thoroughly by spreading on trays in thin layers and exposing to warm air, or in an oven at a temperature not exceeding 60°C. Nowadays drying is usually done by passing warm air upwards through a column of the granules. This 'fluidises' the mass and effects drying at a temperature below the official limit of 60°C.

During drying the granules tend to agglomerate, so the mass is again passed through a sieve to regranulate. If the operations have been carried out properly, only a negligible quantity of fine powder should be present. Some of the granules may be quite small and, from the point of view of uniform weight of tablet, it is actually an advantage to have up to 15 per cent of fine granules present.

### Sieves

No 8 or No 10 sieves may be used for the granulation of the moistened material. After drying, the granules are then passed through a finer sieve. A No 20 sieve is usually satisfactory.

### ALTERNATIVE METHOD

A method for preparing granules for compression into tablets has been devised by Tuerck, Walters and Carkhuff (1960) in which the powders to be granulated are sprayed with granulating fluid while in a rotating coating pan, Fig. 81. The granulating agent is sprayed in the form of a fine mist and the contents of the pan are manipulated by paddles as the pan rotates. By regulating the speed of the pan and the quantity of granulating agent small spherical granules are produced and dried, using hot air while still rotating in the pan. The product is sifted in an oscillating granulator with a No 10 mesh. The authors make a distinction between low-potency tablets, where the bulk of the material is diluent, such as sugar, when they use water as the granulating agent, and cases where the medicament

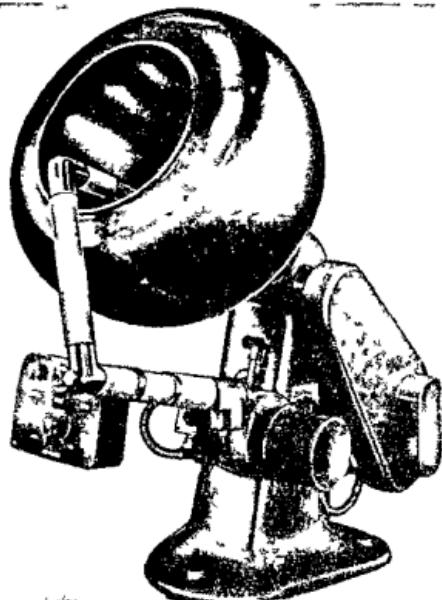


Fig. 81 TABLET COATING PAN  
(Courtesy Manesty Machines Ltd Liverpool)

constitutes the bulk of the tablet, when starch, mucilage or similar granulating agent is used.

### Dry Extracts

Some extracts are prepared in granular form by manufacturers. Dry Extracts of Cascara Sagrada, for example, which is used almost exclusively in tablet form, is prepared as follows. The percolate is concentrated under atmospheric pressure to form a treacly liquid and the pressure is then quickly reduced. The sudden reduction in pressure causes the semi solid to swell up and form a light porous mass, which, when evaporation is complete, is readily crushed to give granules suitable for compression.

### Hygroscopic Substances

These cause difficulties which may be overcome by adding a small percentage of inert powder, e.g. starch or lactose, to the dry material just before compressing.

### Volatile Oils

If volatile oils were added to the mass before granulating and drying, much of the oil would be lost. They may be mixed with the prepared granules

before compression. This can be done satisfactorily by spraying a solution of the oils in alcohol on to the dry granules before compression.

#### LARGE SCALE METHOD

The description of moist granulation so far has been confined to small scale production. On a larger scale the stages of the process are similar except that each stage in the process is mechanized.

It is important to realize that, if results are to be reproducible, each part of the process must be carried out under careful control. For example, even before mixing the ingredients it is necessary to ensure that all powders have been reduced to the most suitable particle size. This is a matter of experience and may differ from one material to another. Generally, fine powders are most satisfactory when several substances have to be mixed together. It is important, to achieve uniformity in successive batches of the same formulation, that particle size should be controlled within narrow limits. The same careful control must be applied to all successive stages in the process.

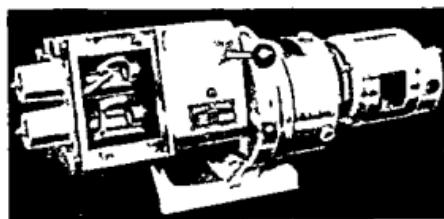


Fig. 8.2 LABORATORY MIXER  
(Courtesy Baker Perkins Ltd., Peterborough)

**Mixing** This is usually done in a mixing machine of a type similar to that illustrated in Fig. 8.2, which is a laboratory model. At this stage, in addition to medicaments, disintegrating agents or binders may be added. Sometimes even a lubricant, such as stearic acid in a volatile solvent, is added. This avoids the final addition of lubricant by tumbling which may cause certain granules to crumble excessively.

**Addition of Granulating Agent** The same type of mixer may be used to convert the powders into a coherent mass by adding the selected granulating agent. Once again it should be emphasised that the same granulating agent or blend of these must be used in precise quantities and at the same temperature to achieve predictable results. When the mix is suitably coherent it is transferred to an oscillating granulator, Fig. 8.3 or to a comminuting machine, Fig. 8.4.

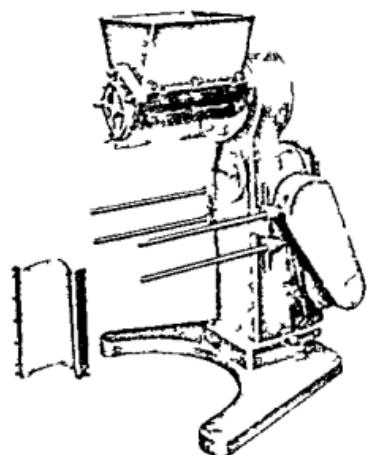


Fig. 8.3 OSCILLATING GRANULATOR  
(Courtesy Manesty Machines Ltd., Liverpool)

**Granulating** The oscillating granulator consists of a hopper leading to a chamber containing a powerful oscillating rotor which forces the material through a screen. Screens are of various sizes No. 8 mesh being commonly used. All parts coming into contact with the materials are of stainless steel. The comminuting machine Fig. 8.4 works on a different principle. It consists of a chamber through which passes an axle carrying a number of swinging blades, sharp on one edge and flat on the other. The blades are capable of speeds up to 5 000 revolutions per minute. The mass is fed to the chamber by means of

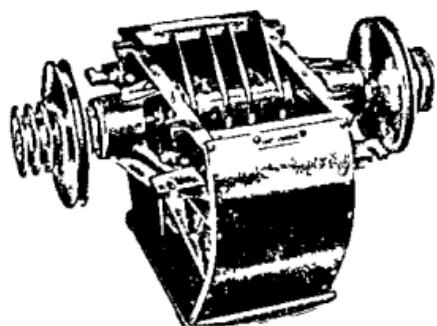


Fig. 8.4 COMMINUTING MACHINE  
(Courtesy Manesty Machines Ltd., Liverpool)

a hopper and is cut up by the swinging blades which are rotated so that the knife edges are foremost. The granules formed fall through a screen at the base of the chamber. The size of screens is of less importance in this process since the granules are formed in the comminuting chamber.

**Drying** The granules are dried at a temperature not exceeding 60°C on trays in ovens or by means of warm air in 'fluidised' columns as mentioned on p 99.

**Resifting** The oscillating granulator is again brought into use, this time with a finer sieve, to obtain the granules in the correct particle size. No 16 or No 20 sieves may be used at this stage.

**Final Additions** A lubricant is usually added to the granules at this stage by gently tumbling the granules with the lubricant in a drum mixer.

All weights having been carefully checked throughout the process the granules are ready for compression.

## GRANULATION BY PRELIMINARY COMPRESSION

'Double Compression,' 'precompression' and 'slugging' are terms also used to describe this process. It is done by first compressing the dry powder into large tablets or 'slugs' as they are called, by means of a tablet machine adapted for powder feeding. The slugs are then broken up into suitable granules in an oscillating granulator.

It has been said that fine powders are not suitable for compression into tablets, but for this purpose the tablets need not be perfect so long as they are hard. The use of large dies and punches assists in feeding the powder to the die, and, to ensure uniform feeding, excess powder is fed into the die and the excess scraped away by means of a mechanical device. Moreover, compression in this machine is gradual in order to allow entrapped air to escape more readily. Compression into slugs is apt to put an excessive strain on a machine and, since the tablets may be broken and compressed pieces gain access to the dies, a device is incorporated to 'cushion' any excess pressure.

By this process, two possible causes of decomposition are avoided, namely, moisture and heat. In addition, there is little loss of volatile matter.

## 2 Binders

Some substances, when granulated with water, alcohol, or other simple solvent, tend to produce granules which break down too easily into a powder. In such cases a 'binder' is used. Normally the binder is included with the granulating agent in solution, and this is the most satisfactory method. Among

the granulating agents quoted, for example, the following serve also as binders—acacia, tragacanth, gelatin, sucrose and glucose, 20 per cent mucilage of acacia, for example, is a most useful binding and granulating agent. When used dry, the quantity of binder may vary between 2 and 10 per cent.

Acacia and tragacanth (2 to 3 per cent) are used as binders for 'lozenge' tablets, as they are effective in producing hard masses.

## 3. Lubricants

### Picking

During compression there is a tendency for the granules to adhere to the surface of the punches. This is called *picking*. The resulting tablets show a pitted surface instead of the normal smooth surface. Picking may be caused by scratches on the punches, or by using a damp granulation, but it is very liable to occur when these causes are corrected. It may be prevented by adding a lubricant to the granules. The most satisfactory lubricants are the stearates, magnesium stearate (½ to 1 per cent) probably being the most popular. Calcium sodium and zinc stearates and stearic acid have also been used in similar proportions.

Purified talc (1 to 2 per cent) is less efficient although it is still commonly used.

Liquid Paraffin (1 per cent) and boric acid (½ to 2 per cent) are also used, the latter being useful for solution tablets for external use since a clear solution is produced.

It should also be mentioned that the use of chromium-plated punches or dies, or the use of special metals such as phosphor bronze for their construction, has been found effective in counteracting picking.

In most cases, a lubricant is necessary to avoid picking, but some substances, e.g. potassium iodide and potassium bromide, may be compressed without one if the precaution is taken to use suitable punches and dies, and to compress the granules immediately after drying.

**Binding** Another primary function of the lubricant is to reduce *binding*, i.e. the friction between the die wall and the lower punch.

**Flow Properties** Attention to the proper lubrication of the granules also assists their free flowing, and ensures tablets of a much smoother and more polished appearance. The flow properties of powders and the effect of added lubricants may be investigated by the measurement of the *angle of repose*, see p 118, and Train (1958). Craik and Miller (1958) used this method to examine the flow properties of different kinds of powders with varying

moisture content and measured the effect of adding small quantities of magnesium oxide. They offer an explanation of adhesion between particles by intermolecular forces and by the surface tension of moisture films. The added magnesium oxide is finely divided and is adsorbed on the powder particles, causing reduction of adhesion between particles, and improving flow properties. The adhesion is said to be overcome because of the size of the magnesium oxide particles compared with the short range of the intermolecular forces causing adhesion. Adhesion due to surface moisture films is also prevented by the magnesium oxide particles preventing the continuity of the films.

#### 4 Disintegrating Agents

Tablets consisting entirely of insoluble substances would, in many cases, fail to disintegrate when swallowed. To ensure the tablet breaking up in the stomach, a disintegrating agent is used.

Three types are in use—

- 1 Substances which swell up on contact with moisture
- 2 Substances which melt at body temperature
- 3 Substances which react, with effervescence, on contact with moisture

Starches are examples of the first type, maize and potato starch being commonly used. This type of disintegrating agent is used in most cases.

Of the second type, cocoa butter is an example. This is used in the form of a solution in ether.

Disintegration may be effected by adding a small quantity of tartaric acid to half the material and a chemically equivalent amount of bicarbonate of soda to the remainder. The two parts are granulated and dried separately, and then mixed before compression. The effervescence which occurs on contact with moisture in the stomach causes disintegration.

Wetting agents may also be used to hasten disintegration. Cooper and Brecht (1957) give an account of the use of various surface-active agents for this purpose.

#### 5 Diluents

If the weight of medicament in each tablet is very small, a quantity of an inert substance such as lactose is added to increase the bulk. A reference to minimal size is made later when dealing with standardisation.

The selection of a suitable diluent is generally left to the pharmacist, but the Pharmacopoeia gives the formula for one diluent, viz. *Chocolate Basis*.

### FORMULATION

Substances in the form of small crystals will require no additions apart from the probable use of a lubricant, but in other substances it may be necessary

to include the adjuncts already mentioned. Table 8.2 will assist in devising a formula—

Table 8.2

Adjunct	Reasons for including	Material used	Percentage
Diluent	To increase bulk to a suitable size	Sucrose, lactose, sodium chloride etc	A sufficient quantity
Granulating agent	To convert fine powders to granules. Usually used in conjunction with a binder	Water, alcohol, gum and starch mucilages, solutions of glucose, sucrose etc	As above
Binder	Used with fine powders to form granules which will not crumble	Powdered acacia and tragacanth	1 to 3
Lubricant	To prevent picking to assist 'flow' of granules and improve appearance of tablets. Used with most tablets	Gelatin, glucose, sucrose, etc Magnesium stearate Purified talc Stearic acid. Liquid paraffin. Boric acid etc.	2 to 10 ½ to 1 1 to 2 ½ to 2 Up to 1
Disintegrating agent	To ensure the crumbling of the tablet when swallowed. Necessary with insoluble substances which do not contain starch	Potato starch or other starch Alginic acid	Small percentages 5-10

**EXERCISE 8.1**

Devise a suitable formula for, and prepare, 100 tablets, each containing 5 grains of Potassium Bromide

5 grains of Potassium Bromide is a satisfactory bulk, therefore no diluent is necessary

The salt occurs in crystals, therefore no granulating agent or binder is necessary

Picking may occur and may be prevented by the use of a lubricant

The salt is readily soluble in water and is intended to be dissolved in water for administration, therefore no disintegrating agent is required

*Formula*

Potassium Bromide	600 gr
Magnesium Stearate	1½ gr

Pass an excess of the salt through a No 16 sieve, shake over a No 30 sieve and reject the powder which passes through. Weigh 600 grains of the granules and shake in a dry bottle with the magnesium stearate and compress. The excess is necessary to allow for one or two trial tablets and for any faulty ones

*Note* Tablets of potassium bromide, potassium iodide and some other soluble crystalline substances may be made without a lubricant provided the crystals are dried in a warm atmosphere immediately before compressing

**EXERCISE 8.2**

Give suitable formula for, and prepare, 100 tablets, each containing 5 grains of Aspirin

5 grains of aspirin is a satisfactory bulk, therefore no diluent is necessary

Aspirin may be purchased in the form of small dense granules, therefore no binder or granulating agent is required

Picking may occur, therefore a lubricant is added

Aspirin is insoluble, therefore a disintegrating agent is indicated

*Formula*

Aspirin	600 gr
Purified Talc	12 gr
Starch	30 gr

Shake the Aspirin with the talc and starch in a dry bottle and compress

**EXERCISE 8.3**

Devise a suitable formula for, and prepare, 100 tablets, each containing—

Rhubarb	3 gr
Sodium Bicarbonate	1½ gr
Ginger	½ gr

The medicaments add up to a total of 5 grains, so there is no need for a diluent

The materials are in the form of fine powders, therefore both binder and granulating agent are necessary. In the presence of aqueous alkali a reddish colour develops and tablets of an unsatisfactory appearance are produced. Tablets may be made using a small percentage of acacia and granulating with acetone but although the colour is satisfactory, the

(continued overleaf)

*Exercise 8.3 continued*

tablets have poor mechanical strength Excellent granules may be made by pre-compression

*Picking may occur, therefore a lubricant is necessary*

Bicarbonate of soda is soluble, and both rhubarb and ginger contain starch, therefore no disintegrating agent is required

*Formula*

Rhubarb	360 gr
Sodium Bicarbonate	180 gr
Ginger	60 gr
Purified Talc	12 gr

Compress the mixed powders into hard tablets Carefully break and screen to form suitable granules Shake the granules with the talc in a dry bottle and compress

**EXERCISE 8.4**

Sodium Bicarbonate	500 gr
Oil of Peppermint	10 minims
Make into 100 tablets	

Once again, a diluent is unnecessary

These are 'lozenge' tablets and are to be prepared from a fine powder, therefore a granulating agent with good binding properties is called for A 20 per cent solution of acacia would be satisfactory, especially if combined with a binder, e.g. 3 per cent powdered acacia

*Disintegration is to be avoided, therefore no disintegrating agent is used.*

*Picking may occur, therefore a lubricant is added.*

*Formula*

Sodium Bicarbonate	600 gr
Oil of Peppermint	12 minims
Powdered Acacia	18 gr
Solution of Acacia (20%)	q.s
Magnesium Stearate	1½ gr

Mix the bicarbonate of soda with the powdered acacia and granulate with the solution of acacia When dry add the oil of peppermint This is done by dissolving the oil in a little alcohol and spraying it on the dry granules, which are continually turned over during the process Finally, add the magnesium stearate and shake the whole in a dry bottle

**EXERCISE 8.5**

Devise a suitable formula for, and prepare 100 tablets, each containing  $\frac{1}{10}$ th grain of Strychnine Sulphate

The bulk of medicament is too small, therefore a diluent is used Lactose, sufficient to make a tablet weighing about 1 grain, would be satisfactory

The triturate of strychnine sulphate and lactose is in fine powder, therefore a granulating agent and a binder are necessary

*Picking may occur, therefore a lubricant is called for*

The salt and lactose are both soluble, therefore no disintegrating agent is necessary

*Formula*

Strychnine Sulphate	4 gr
Lactose	120 gr
Magnesium Stearate	1 gr
Starch Mucilage	q.s

Add the lactose gradually to the strychnine sulphate with careful trituration until thoroughly mixed  
 Granulate with the mucilage using a No 20 sieve  
 Dry and pass the granules through a No 20 sieve  
 Shake the granules with the talc in a dry bottle and compress

**EXERCISE 8.6**

Devise a formula for, and prepare, 500 tablets, each containing 1 grain of Phenobarbitone

Compressed tablets of phenobarbitone sometimes do not disintegrate readily and the official disintegration time is half an hour. Berry and Ridout (1950) found that a satisfactory tablet disintegrating in the normal 15-minute period could be made using alginic acid as disintegrating agent. The quantity used is 10 per cent and, since the finely powdered alginic acid tends to separate from the granules in the hopper of the machine, it is incorporated in the granules.

Since the medicament is in the form of fine powder a granulating agent and binder are required

Picking may occur, therefore a lubricant must be added.

*Formula*

Phenobarbitone	600 gr
Alginic acid	60 gr
Stearic acid	6 gr
Solution of sucrose 50% w/v	q.s

Mix the finely powdered phenobarbitone and the alginic acid and granulate with the sucrose solution. When dry shake the granules with the stearic acid in a dry wide-mouthed bottle and compress

**Hypodermic Tablets**

These may be made by moulding (see p 85) or by compression. In either case strict aseptic procedure is necessary, but considerable difficulty is experienced when tablets are made by compression. Stephenson (1950) describes the various precautions that are taken. These include air conditioning. The air is filtered so that gross contamination is reduced in the

laboratory. The preparation of materials is done in special cabinets supplied with sterile air under positive pressure. All parts of the machines which come into contact with the granules must be sterilised and other parts flamed and treated with an antiseptic followed by sterile oil at the beginning of each day's work. Packaging is done in cabinets similar to those used for the preparation of materials (See also Chapter 29.)

**COMPRESSION**

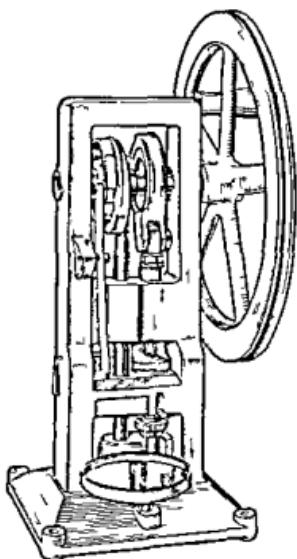
The following practical details and sketches refer to a small single-punch hand machine. Some belt-driven machines are of a similar type, but the large rotary, multi-punch machines operate on a different principle and will be described in outline later.

**HAND-OPERATED MACHINES**

The accompanying diagrams (Figs. 8.5, 8.6 and 8.7) illustrate the manner in which the machine operates, and will repay careful study. Several movements take place at one time and the cycle of movements should be followed.

**CYCLE OF MOVEMENTS**

- 1 The upper punch rises to allow the hopper shoe to move over the die.
- 2 The lower punch drops and granules feed from the shoe into the die. A shaking movement of the shoe assists the flow of granules.
- 3 The shoe moves aside and the upper punch drops, compressing the granules into a tablet.
- 4 The upper punch rises clear and the lower punch rises flush with the top of the die, ejecting the tablet.



**Fig. 8.5 HAND OPERATED TABLET MACHINE**  
Capacity regulating screw is concealed by the circular tray  
(Courtesy Manesty Machines Ltd.)

- 5 The hopper shoe again moves forward over the die, pushing aside the newly compressed tablet
- 6 The lower punch drops and cycle restarts

#### ADJUSTMENT OF THE MACHINE

##### For Capacity

- 1 The lower punch is set at a low position by moving the capacity regulating screw upwards and turning the driving wheel until the punch is at its lowest point
- 2 A weighed quantity of granules is placed in the die
- 3 The lower punch is then moved upwards by turning the driving wheel until the surface of the granules is level with surface of die
- 4 The lower punch is now in its correct dropped position and is fixed by moving the capacity regulating screw downwards until it presses against the base of the machine

##### For Ejection of Tablets

- 5 The lower punch is raised as far as it will go by turning the driving wheel. The top of the punch

should now be level with the upper surface of the die. If it is not, the ejection regulating screw should be moved up or down until it is so.

##### For Compression

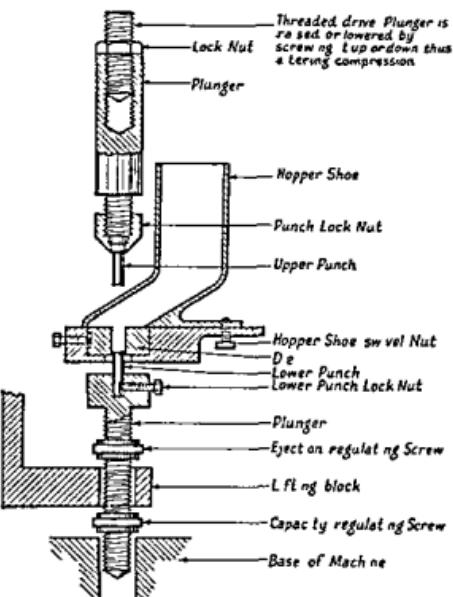
- 6 The upper punch is first set high to give very light compression. A tablet is made and examined. Compression is increased, tablet by tablet, until satisfactory. A rough test of the mechanical strength of a tablet may be made by dropping it from a height of one to two feet, when it should not break.

##### Checking Adjustments

- 7 A few tablets are now made and weighed. If the weight is not correct, the necessary adjustments are made by raising or lowering the capacity-regulating screw and correcting the compression.

##### Locking in Position

- 8 When the above adjustments are correctly completed, the adjusting screws are locked in position.



**Fig. 8.6 DIAGRAM OF PART OF SINGLE PUNCH HAND TABLET MACHINE**

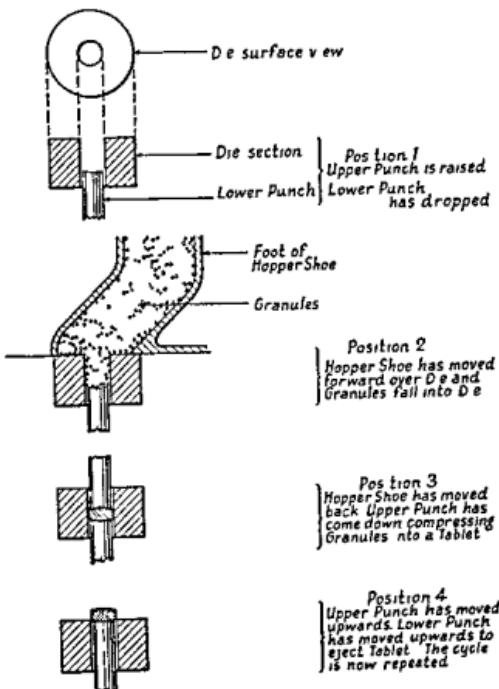


Fig. 8.7 MOVEMENTS INVOLVED IN COMPRESSION

and compression proceeded with. When making a large batch of tablets, it is necessary to check at regular intervals during compression.

#### CARE OF THE MACHINE

It need scarcely be said that the machine should be kept clean and well oiled, but certain other precautions should be taken.

When compressing the driving wheel should always be turned in a clockwise direction. If the drive is reversed, the die may be filled twice and the machine will jam. If this occurs, the hopper shoe must be removed and the driving handle turned backwards to eject the double tablet.

When a batch of tablets has been made, the machine should not be left in an unclean condition. The punches and dies should be removed and thoroughly cleaned. It is a good plan to smear them with a little oil until they are required again for use.

#### ROTARY TABLET MACHINES

In these machines (Fig. 8.8) there is a circular rotating head consisting of three parts—

- (a) an upper part carrying the upper punches,
- (b) a central part carrying the dies,
- (c) a lower part carrying the lower punches

The hopper delivers the granules to the 'feed frame' placed over the central part containing the dies. The object of the feed frame is to confine the granules in position immediately over the die track. As the head revolves, the dies come under the feed frame in succession and are filled. Compression is effected by the upper and lower punches passing

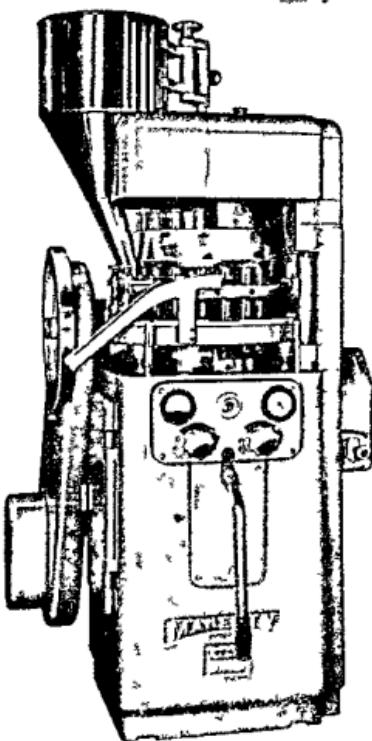
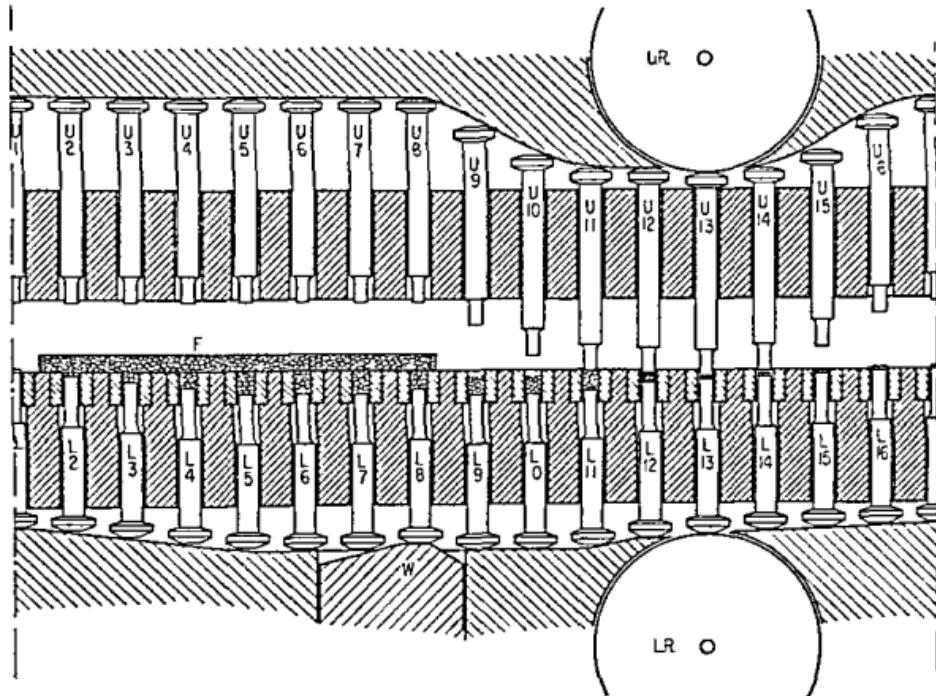


Fig. 8.8 MANESTY DX2 ROTARY TABLET MACHINE

(Courtesy Manesty Machines Ltd. Liverpool)



**Fig. 89. DIAGRAM OF PUNCH TRACKS OF ROTARY TABLET MACHINE OPENED OUT TO SHOW CYCLE OF ONE REVOLUTION** *U R*—UPPER ROLLER *L R*—LOWER ROLLER *H*—CAPACITY ADJUSTER  
*F*—FEED FRAME WITH GRANULES

*U1* to *U8* Upper punches in raised position

*L1* Lower punch at top position tablet ejected.

*L2* to *L7* Lower punches dropping to lowest position and filling die with granules to an overfill at *L7*

*LB* Lower punch raised to expel excess granules giving correct capacity  
*U9* to *U12* Upper punches lowering to enter die at *U12*

*L9* to *L12* Synchronised with *U9* to *U12* lower punches rising prior to compression.

*L13* and *U13* Upper and lower punches pass between rollers and granules are compressed to a tablet

*U14* to *U16* Lower punch rising to completely eject tablet at *U16*

*U1* and *L1* Beginning of cycle

between rollers which gradually compress the granules

The cycle of events in one revolution of the rotary head is shown diagrammatically in Fig. 89 where the circular punch track is shown 'opened out.'

An overload mechanism is incorporated in these machines to 'cushion' excess strain due to the possibility of foreign matter entering the dies or excessive pressure being applied

#### TABLETTING WITHOUT GRANULATION

The F J Stokes Corporation of Philadelphia have recently developed a device, for use with rotary

tablet machines, which feeds powdered material directly to the dies under slight pressure. The device and its operation and uses have been described by Kibbe (1961). The method eliminates the need for granulation with its accompanying use of time and equipment. The device takes the place of the conventional feed frame on the rotary machine. It consists of a floating shoe fixed to a feed frame to carry the powder to the dies. A compressed air power unit transmits to the shoe an orbital movement in a horizontal plane immediately over the die track. As the rotary head carrying the die revolves under it, the shoe rotates, pushing the powder into the dies. The relative movements of the rotary

head and feed shoe, and the shape of the latter, develops sufficient pressure on the material to force it into the dies.

## STANDARDISATION OF COMPRESSED TABLETS

Early methods of standardisation were made, or omitted, at the discretion of the maker, and in many cases no serious attempt was made to ensure uniformity. The result was that many tablets on the market were extremely unsatisfactory, the weight, size, shape and rate of disintegration varying considerably in batches of tablets from different manufacturers.

A considerable amount of investigation into these problems has, however, been carried out in recent years. In 1934 the Swiss Pharmacopœia V introduced standards for weights of tablets and, in addition, a simple disintegration test. Since that time, statistical evidence has been collected relating to the control of weight and content of tablets, and several methods have been proposed for the measurement of the rate of disintegration. As a result of some of this work, standards were first adopted in the *British Pharmacopœia 1932, Addendum VII 1945*.

The following variables have been investigated by various workers with a view to standardisation—

- 1 Shape
- 2 Weight of tablet
- 3 Percentage of medicament
- 4 Rate of disintegration
- 5 Mechanical strength
- 6 Diameter

Standards for 1 to 4 and 6 have been adopted officially and, in addition, colouring and labeling have been defined.

### 1. Shape

It should be noted that *shape* is defined in the *Pharmacopœia* as 'circular with either flat or bi-convex faces'.

### 2. Weight

Small variations in the weights of individual tablets are inevitable and admissible, and, accordingly, accepted limits are officially specified for uncoated tablets.

It should be understood that, since the *size* of tablets has not been standardised, the limits are based on *average weight in the batch under examination*.

A number of these machines is in routine use in the U.S.A., and their advantages are obvious since they are able to eliminate all the stages in granulation.

### *The Official Standards*

In the official test 20 tablets are used. If 20 are not available, 10 are used.

The 20 tablets are weighed individually and the average weight calculated. This average weight could not be accepted as sufficient evidence of uniformity, since single tablets, of wide variation in weight, might give a satisfactory average figure.

Hence, deviations from the average weight are controlled according to the official table, Table 8.3

Average weight	Percentage deviation
0.12 G or less	±10
More than 0.12 G and less than 0.3 G	±7.5
0.3 G or more	±5

Not more than 2 tablets may fall outside this range. If only 10 tablets are available, only 1 may fall outside the range.

In no case is the test considered satisfactory if more than 1 tablet exceeds the average weight by more than twice the specified deviation.

### 3. Percentage of Medicament

Details of the individual assay processes are included in the individual monographs, and percentage limits for the medicaments are stated in each case.

It should be realised that variations in the percentage of medicament, as shown by the result of an assay process, may occur for various reasons, e.g.

- 1 Limits of accuracy in preparing granules
- 2 Variation in the weight of the tablets
- 3 Permitted variation in the purity of the drug
- 4 Errors of 'random sampling'
- 5 Limits of accuracy in the analysis

Reasonable variations are, therefore, allowed for in the official tests.

The assay limits are based on the fact that 20 tablets are used in the tests.

The official limits are widened if fewer than 20 tablets are used in the tests. The official table (Table 8.4) states the permitted variations.

*Table 84*  
**Variation of Standards**  
 (To apply when the stated limits are between 90 and 110%)

<i>Weight of drug in each tablet</i>	<i>Subtract from the lower limits for samples of</i>			<i>Add to the upper limits for samples of</i>		
	15	10	5	15	10	5
0.12 G or less	0.2	0.7	1.6	0.3	0.8	1.8
More than 0.12 G and less than 0.3 G	0.2	0.5	1.2	0.3	0.6	1.5
0.3 G or more	0.1	0.2	0.8	0.2	0.4	1.0

It will be noted that these variations are allowed when the stated limits are between 90 and 110 per cent.

If the stated limits are wider than this a proportionately wider variation is allowed. No method for calculating this proportion is given, but Hadgraft (1945) suggests the following ratio taking Glyceryl Trinitrate Tablets as an example which have limits of 85 and 115 per cent.

The official lower limit allowance, using 5 tablets, is 1.6. But since 85 per cent is lower than 90 per cent, a proportionately greater allowance must be made, viz.

Increased allowance is to  $(100 - 85)$

as 1.6 is to  $(100 - 90)$ ,

$$\text{i.e. the increased allowance} = \frac{1.6(100 - 85)}{(100 - 90)} = 2.4$$

#### 4 Rate of Disintegration

Before discussing the official disintegration test, certain aspects of tablet disintegration must be considered.

##### *Types of Tablet in Relation to Disintegration*

- (a) Tablets for slow solution in the mouth. These are really lozenges, though made by compression e.g. Soda Mint Tablets
- (b) Tablets which are to be masticated, e.g. Phenolphthalein Tablets
- (c) Tablets to be dissolved in water for administration, e.g. Potassium Bromide Tablets
- (d) Buccal tablets which dissolve or disintegrate slowly in the mouth (buccal pouch), and sublingual tablets which are placed under the tongue and slowly dissolve there
- (e) Tablets to be swallowed, e.g. Aspirin Tablets

Types (a), (b) and (d) do not come within the scope of a disintegration test for obvious reasons.

Type (c) comprises tablets which are readily soluble in water and may be crushed to effect solution, therefore no disintegration test applies. There may be exceptions to this, as in the case of Sodium Citrate Tablets, which are added to milk for feeding infants and invalids and where rapid solution is required, and a disintegration test is officially prescribed.

Type (e) includes the majority of tablets, and here the test is very important. This type is now considered further.

##### *How Quickly Should a Tablet Disintegrate?*

Tablets which will disintegrate in a few seconds, or in a few hours may be prepared. Generally speaking, such extremes should be avoided, the first because the tablet may break up in the mouth, and the second because the effect may be unduly delayed.

It has been shown, by X-ray examination of swallowed barium sulphate tablets, that tablets remain in the stomach, on an average, for about four hours before passing into the intestines. In some cases, therefore, it might be satisfactory if the tablet disintegrated within this period, but, generally, a much shorter time limit is preferable.

For most tablets, a disintegration time of 30 minutes would not be excessive, while for others, where a rapid effect is desired, a limit of 1 minute might be more suitable.

However, since it has not been found practicable or considered necessary to introduce a range of exact disintegration times for individual tablets, the Pharmacopœia has adopted a general limit of 15 minutes, within which time disintegration should take place. Such tablets will be satisfactory and will generally break up in a shorter time.

In certain cases, a period of 30 minutes is allowed, e.g. Phenobarbitone Tablets, where difficulty is experienced in making a tablet that will break up readily Sodium Citrate Tablets, which are intended to be dissolved in a little water for addition to an infant's milk feed, should dissolve rapidly for convenience. For these, therefore, the official disintegration limit is 3 minutes.

#### THE OFFICIAL DISINTEGRATION TEST

This test is standardised in the following ways—

- Water is used at 35°C to 39°C.
- A regular degree of movement is involved.
- If necessary, a slight pressure is applied to the tablets.
- Disintegration is judged on final particle size.
- A time limit is involved.

The essential apparatus is a glass or plastic cylinder closed at its lower end by a rustless wire gauze (No 10 sieve). This tube is suspended in water at 35° to 39°C, the temperature being maintained thermostatically, Fig. 8 10(a). The tube is raised and lowered at a constant rate (30 times per minute) through a distance of 75 mm.

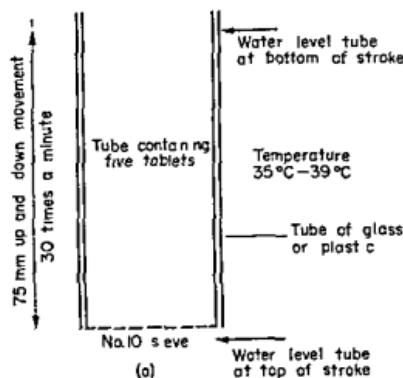


Fig. 8 10  
(a) Diagram of disintegration tests  
(b) Guided disc



At the highest point the wire gauze just breaks the surface of the water thus giving a turbulent effect. At the lowest point the top of the tube does not come below the surface of the water.

Five tablets are placed in the tube for the test. The time for the tablets to disintegrate and pass through the sieve is noted and should comply with the time specified for that tablet in the official monograph.

Some particles may not pass through the sieve and may remain suspended in the water but the tablets are considered to have disintegrated if these particles are small enough to pass through.

If the tablets do not disintegrate the test is repeated using a plastic disc of specified weight which will impart a slight pressure on the tablets. The disc is kept horizontal in the tube by a guide ring supported on three pins, Fig. 8 10(b).

#### DISINTEGRATION TEST FOR ENTERIC COATED TABLETS

This test is carried out in two stages as follows—

- The procedure for ordinary tablets described above is carried out using, instead of water, an aqueous acid pepsin solution containing 0.3 per cent w/v pepsin and 0.6 per cent v/v hydrochloric acid. This solution is intended to simulate the reaction of the stomach fluid, and the tablets should remain intact during a period of three hours. Particles of coating may break away but this may be ignored.
- The tablets are washed by immersing the tube in water, and the first procedure is repeated, this time using an aqueous alkaline solution containing 0.3 per cent pancreatin, 1.5 per cent w/v bicarbonate of soda and 0.5 per cent w/v sodium tauroglycocholate. This solution is intended to simulate the reaction of the intestinal fluid.

The tablets should disintegrate within one hour. If the tablets fail to disintegrate the test is repeated using, in the second stage, the plastic disc mentioned previously.

#### UNOFFICIAL METHODS

A consideration of unofficial methods, which have been suggested from time to time, for the standardisation of disintegration is instructive.

Early methods depended on placing the tablets in warm water and subjecting them to occasional shaking or rotation, e.g. the method adopted by the Swiss Pharmacopoeia V. Since the extent of agitation is a significant factor, such methods are not completely satisfactory.

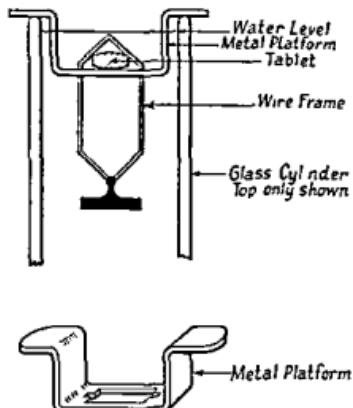


Fig. 8 11

H Berry (1939) devised a novel method in which the tablet, supported at its edges on two sides, has suspended upon it, by means of a wire frame, a 20-G weight (Fig. 8 11). Immersed in water at 37°C, the time is noted for the tablet to break and the weight to fall.

Although apparatus and conditions are standardised, this method is open to the two following criticisms—

- 1 Large crystals or laminated pieces may delay the fall of the weight
- 2 Tablets, on occasion, contain a hard core which may delay the fall of the weight.

A somewhat similar method, published at the same time by Brown, used an adapted balance

#### THE METHOD OF THE B.P. 1953

This test, devised by Berry and Smith (1944), and official in previous Pharmacopoeias, consists in exposing the tablets to the effect of water at 37°C under *standard conditions of agitation*. Disintegration should take place within 15 minutes.

The following are the specifications—

5 tablets are used for each test

Each is placed in a test tube, 6 inches in length and 1 inch in internal diameter.

The tubes contain water, at 37°C, and, when stoppered, contain a half-inch air space.

The tubes are fixed on a rotating device immersed in a water bath, maintained at a temperature of 37°C.

The rotating device, which repeatedly inverts the tubes, is set in motion at such a speed that the tablets fall through the water in the tube, but never strike the ends.

The half inch air space in the tubes gives a turbulence to the contents which is constant, since the amount of air in each tube is similar.

After 15 minutes the contents of the tubes are examined.

All five tablets should have disintegrated.

If one tablet fails to comply with the test, another test may be applied using five tablets from the same batch. All five must disintegrate in the stated time.

Figure 8 12 shows diagrammatically the form of apparatus used.

Two more recent methods, one by Hoyle (1946), and the other by Prance, Stephenson, and Taylor (1946), involve the raising and lowering of the tablet at a constant rate in a tube of water at 37°C by means of a mechanical device. In these, the conditions are standardised, viz. water is used at 37°C and the degree of agitation is constant. These methods are satisfactory and, moreover, the exact time of

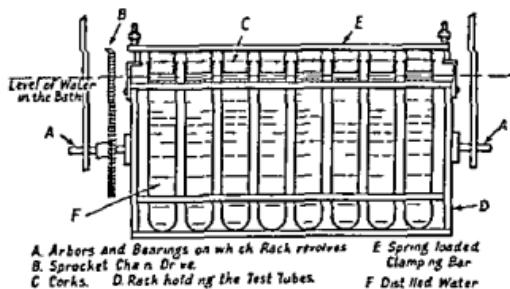


Fig. 8 12

disintegration is measured. This is useful in formulation experiments, and it will be seen that it is essentially the present pharmacopoeial method.

In their tests, some investigators have used fluid, which corresponds with the gastric juice. The evidence available suggests that no significant differences in disintegration times are obtained by the substitution of such liquids for water, except, of course, in testing enteric coated tablets.

### 5 Mechanical Strength

No standards for the mechanical strength of tablets have been adopted by the Pharmacopoeia. This fact is an indication of the difficulties involved in devising a test which will provide the required information.

Manufacturers employ tests to ensure that their tablets will withstand the normal risks of handling, transporting etc., and these are either simple qualitative tests for 'wear and tear' or tests for strength using mechanical devices of some kind. Tests depending on the sensitivity of the fingers in crushing or breaking have been used, presumably since tablets were first made, and this 'feel judgement', as it is sometimes called, can be a good guide to

### Tests on Breaking Strength

One uses a steel spring pressure device, graduated in 0.5 kg and readable to 0.25 kg, designed by the Monsanto Chemical Co Ltd (Fig. 8.13). In this the tablet to be tested is placed between the spindle and anvil, and pressure is applied by turning the knurled knob just sufficiently to hold the tablet in position. The reading of the pointer on the scale is then adjusted to read zero. The pressure is now increased as uniformly as possible until the tablet breaks. The pointer now reads the pressure required to break the tablet. The 'hardness factor' is the average of several determinations. In conjunction with this a density factor is calculated thus:

$$\frac{\text{Weight of tablet (G)}}{\text{Thickness at crown (in)}} = \text{density factor}$$

The instrument does not entirely eliminate the personal factor since the pressure of setting at zero and the rate of the application of pressure can vary. With a little practice, however, fairly concordant results are obtained. The figures will vary with the size and content of the tablets but can form a useful guide to the production of satisfactory products.

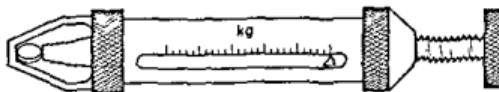


Fig. 8.13 MONSANTO HARDNESS TESTER.

quality. Also, it is quick, and can be used immediately to correct machine adjustment.

To eliminate the personal factor a number of methods have been adopted which fall under three headings—

- 1 Tests on breaking strength
- 2 Abrasion tests
- 3 Penetration tests

In an interesting series of review articles on this subject Nutter Smith (1949) discusses the pros and cons of some of these methods.

Table 8.5 is due to Smith (1949) and shows the kind of results obtained.

Another instrument using a similar principle is the Strong Cobb Hardness Tester (Fig. 8.14). In this apparatus the tablet D is placed on the support E and a pneumatic pressure is built up by pumping the handle B. This lowers the arm C until it touches the tablet. The pressure is gently increased until the tablet breaks when the reading on the scale A is noted.

Shotton and Ganderton (1960) devised a machine to eliminate the personal factor and which increased the load on the tablet at a constant rate. Strain

Table 8.5

	Av Wt (G)	Thickness (in)	Density	Resistance (kg)
Phenobarbitone, B.P., 1 gr	0.080	0.111	0.72	2.16
Acetylsalicylic acid, B.P., 5 gr	0.363	0.160	2.27	2.58
Potassium Bromide, B.P., 5 gr	0.316	0.118	2.68	5.16
Penicillin Lozenges, 500 I.U.	1.140	0.218	5.23	7.96

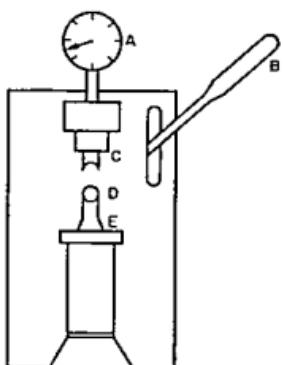


Fig. 814 DIAGRAM OF STRONG COBB HARDNESS TESTER

gauges incorporated in the apparatus measure the applied load.

#### ABRASION TESTS

Rolling or shaking tests of a simple empirical nature have been described by Nutter Smith (1949), and Webster and Van Abbe (1955). These consist of mechanical devices which subject the tablets to shaking or tumbling effects. After a period the abraded material is sifted from the tablets and the percentage estimated.

The amount of powder eroded by such 'wear and tear' tests will vary according to the severity of the treatment, and a limit of abrasion in terms of separated powder decided for each type of apparatus. The speed and period of shaking or tumbling must, of course, be standardised.

An interesting point in connexion with abrasion tests is the effect of the shape of the tablets. Flat tablets with sharp edges show greater effects of 'wear and tear' than flat tablets with chamfered edges, and the most satisfactory shape is bi-convex.

#### PENETRATION TESTS

Smith (1949) described experiments with the Vickers Diamond Hardness Testing Machine. This type of machine makes an indentation under a given load. The impression may be made with a ball or diamond pointed indenter. The extent of the indentation is measured, by area or depth according to the type of machine. Hardness is expressed by the following formula—

$$\frac{\text{Applied load (in kg)}}{\text{Area of indentation (in mm}^2\text{)}} = \text{hardness value}$$

Such machines are made for testing the hardness of metals and are generally too large and too costly for testing such comparatively soft materials as tablets. Machines for testing the rheological properties of plastics, waxes, bitumens etc. may be used to test tablet hardness. Hilger and Watts make a machine with cone and ball indenters. The depth of penetration is recorded directly. It may be read to 0.02 mm, the image being projected on a ground-glass screen. The load varies from 0 to 2,000 G with an accuracy of 0.01 per cent at maximum loading.

#### 6 Diameter

It has already been mentioned that the *gross weight* of official tablets has not been standardised. This means that officially acceptable tablets from different makers may vary in size, according to the quantity of diluent and other adjuncts used in their preparation. This may prove disturbing to a patient who may be supplied with tablets of different size and shape on the same prescription at different times.

Smith (1949) who made a careful investigation of tablet sizes, has pointed out that cases occurred where tablets were found to be two, three, four, and even five times the weight of similar tablets from other makers.

In recent years, however, much useful co-ordination by the Association of British Pharmaceutical Industries has resulted in a large measure of uniformity in tablet sizes. As a result of this the *British Pharmacopoeia* in 1958 introduced standards for the diameters of official tablets. Although gross weight is still unstandardised the control of diameter means that little variation will occur since thickness and diameter are closely related to the production of a tablet of normal and pleasing appearance.

Diameters are given officially in 32nds of an inch, the largest being 20/32nds and the smallest 6/32nds.

Tablets containing different doses of a drug are made in different diameters.—Table 8 6

Table 8 6

	Weight (mg)	Diameter (32nds of in.)
Acetylsalicylic Acid Tablets	150	10
	300	12
	450	14
	600	16

When a drug is prepared in a series of very small doses it is also usual to use diluent to produce tablets with a corresponding series of diameter sizes, Table 8 7

Table 87

	Weight (mg)	Diameter (32nds of in.)
Dienoestrol Tablets	0.1	6
	0.3	7
	1.0	8
	5.0	9

This variation of diameter with dose is a useful visual check but is not always adhered to. Ephedrine Hydrochloride Tablets and Morphine Sulphate Tablets in their two smallest sizes are all of 6/32nds of an inch in diameter, and the three smallest sizes of Amylobarbitone Tablets are of 7/32 of an inch in diameter.

#### PERMITTED VARIATION IN DIAMETER

- An excess in diameter of not more than 1/32nd of an inch is permitted in tablets of 10/32nds of an inch and over.
- A deviation of  $\pm 5$  per cent is allowed generally except for diameters exceeding 16/32 of an inch when a deviation of  $\pm 3$  per cent is allowed.

#### Shape

Other dimensions which are not standardised are—

Thickness at the crown

Thickness at the edge

Curvature

The Pharmacopoeia defines the shape of tablets as flat or bi-convex discs.

With bi-convex tablets it is possible to have a range of curvatures but manufacturers normally restrict curvatures to two, one for plain tablets and the other for tablets which are to be coated. The latter have thin edges for reasons explained under 'Tablet Coating' below.

To produce a tablet of good appearance there is a well-recognised relationship between diameter and thickness. A thickness at the crown of about half the diameter is generally accepted as most satisfactory.

Tablets which are too thin are liable to break easily and those which are too thick may give difficulty in swallowing.

Interesting comments on tablet dimensions are given by Firth (1948) and Smith (1949).

### TABLET COATING

Two methods are used—

- Pan Coating
- Press Coating

#### (a) Pan coating

This is the traditional method of sugar coating, and it is carried out in a rotating copper pan (Fig. 81) into which warm or cold air can be blown. Practice varies somewhat but the following procedure is commonly used.

While the tablets are tumbled in the rotating pan, small quantities of syrup are added. The syrup may consist of sucrose 2 parts and water 1 part. After each quantity of syrup has become evenly dispersed over the surface of the tablets and dried under the warm air current a little dusting powder is added. During the addition of the dusting powder the air current is switched off. The composition of the dusting powder varies but a suitable formula consists of—

Starch	50 parts
Talc	10 parts
Acacia	1 part

During the addition of the syrup it may be necessary to prevent the tablets from sticking together in masses by hand manipulation.

The alternate additions of syrup and dusting powder are repeated until a suitable coating has been built up. Towards the end of the process, warm syrup without powder is used to obtain a smooth surface, and finally cold syrup is applied in one or two portions and dried by cold air. This ensures a hard coat. The rotation must be stopped immediately the tablet coating is dry, otherwise powdering of the surface occurs.

The tablets are now polished in a similar pan coated with a thin layer of about  $\frac{1}{8}$  in. of paraffin wax. The tablets acquire a film of wax as they roll in the pan, the polishing process taking about twenty minutes. The whole process of coating a batch of tablets takes several days.

It is essential that not less than a satisfactory minimum weight of tablets is coated in one batch as the mutual rubbing under a reasonable weight of tablets is necessary to spread the coating evenly. Small experimental coating pans of approximately 16 in. in diameter require a few pounds of tablets, a 30-in. pan will deal with about 30 lb of tablets. It must also be remembered that the weight of the finished tablets will be about twice the weight of the uncoated tablets.

Tablets for pan coating are usually made with thin edges, e.g. in Fig. 81(b) rather than (a). The

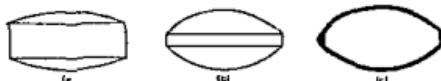


Fig. 8.15

advantages of this are that the coating can be applied more evenly, particularly at the edges (c). As a result, the final weight and size of the tablet is not unduly increased. There is also a saving in time and material.

Coloured tablets are, of course, produced by the use of coloured syrups.

#### (b) Press Coating

The principle of this process is that a layer of dry coating material is pressed onto a preformed tablet. This is done by placing the tablet to be coated (the core tablet) in the die of a tablet machine, in which is a layer of coating material in granular form, covering the tablet with another layer of coating granules and compressing to form a skin round the tablet.

The idea is not new, a British patent having been taken out as early as 1896. Whitehouse (1954) gives an account of some of the earlier processes.

Two types of press coating machines in use in this country are the Prescooter introduced by Evans Medical Supplies Ltd., of Speke, Liverpool (White-

house 1954) and the DryCota of Manesty Machines Ltd., also of Speke, Liverpool (Anon 1955).

In the Prescooter, core tablets are fed from a hopper to the dies of the press coating machine in which there is already a layer of coating granules. A mechanical device centres the tablets and another layer of coating granules is introduced above them. The punches then compress the coating on to the tablets. It is claimed that an interval between preparing the core tablet and applying the coat is desirable for the following reasons—

A day or two are required for the core tablet to attain stable dimensions.

Export tablets may require a sub-coat of varnish before applying the final dry coat.

The core tablet may be analysed and 'passed' before coating, thus avoiding possible waste.

In the DryCota, two rotary tablet machines are used side by side, one to make the core tablets and the other to apply the coating. The coating process follows immediately after the core tablet compression. It is claimed that by this method contamination is avoided and that soft core tablets can be used. A transfer mechanism, situated between the two machines, carries the core tablets to the coating machine. This mechanism consists of radially disposed fingers each supporting a transfer cup and locating with the dies of each machine. The newly

Table 8.9

#### A Comparison of Pan Coating and Press Coating

Pan coating	Press coating
1 Aqueous sugar solutions are used which may decompose some medicaments unless a sub-coat of varnish is used.	No moisture used, therefore, no hydrolytic changes.
2 Tablets must be hard to withstand tumbling in the coating pan, therefore disintegration may be delayed.	Tablets need not be hard.
3 A lengthy process, each batch taking several days.	A relatively quick process.
4 A "batch" process, therefore, small quantities take as long as large quantities.	A continuous process. Small quantities take a relatively short time.
5 A skilled process requiring considerable attention.	A mechanical process proceeding automatically and depending on sound granule formulation and machine maintenance.
6 Final polishing required.	No final polishing required.
7 Coating is mainly sugar.	A wider choice of coating materials and, therefore, the possibility of disintegrating agents in the coat.
8 Embossing on tablet surface not practicable.	Embossing designs possible.
9 Multi-layer tablets not possible.	Multi-layer tablets possible (Gwilt 1960). Coating may contain a medicament incompatible with the content of the core tablet.

compressed core-tablet is picked up by a finger and carried to the coating machine. The transfer unit and rotating turret of the coating machine travel at the same speed, and for a short period each finger is immediately over a die. During this time the finger places the tablet in the centre of the die, which already contains a layer of coating granules. A second layer is now added and the coating is compressed on the tablet.

#### TABLET COATINGS AND OTHER METHODS FOR THE CONTROLLED RELEASE OF ORAL DRUGS

Enteric coating has already been described in Chapter 5. Enteric coating is one example of a coating which controls the time of release of a drug from a pill, tablet or capsule. In this case its particular aim is to protect the drug in its passage through the stomach and release it in the intestine where its effect is required.

In recent years many attempts have been made to use this principle of *delayed release* in order to maintain an effective concentration of drug in the blood over a satisfactory period. The traditional method of taking a dose several times a day results in 'peaks and hollows' in the blood concentration of the drug. The use of *multi layer tablets* in which each successive layer is released some time after the preceding one is convenient, in that possibly only one dose per day is required. It is, however, a repeated dose and suffers from the same disadvantage as the 'thrice daily' dose in that the blood concentration is not constant. During the periods between the release of the drug its concentration in the blood will fall to ineffective levels.

#### ORAL DEPOT THERAPY

In order to maintain a satisfactory concentration in the blood the regular release of drug must be sufficient to replace losses due to excretion or breakdown. This sustained effect is called oral depot therapy since, in effect, the tablet or capsule acts as a depot releasing the drug.

A number of ingenious methods have been devised to achieve this aim including the following—

- 1 Capsules or tablets containing numerous coated pellets which release a drug successively and continuously over a prolonged period
- 2 Ion exchange resins, combined with drugs which are exchanged with ions in the alimentary tract. This takes place continuously as the insoluble resin passes through the tract
- 3 Tablets in which the drug is contained in a fatty

or waxy core and from which it is released continuously by the erosion of the tablet

- 4 Tablets with a porous plastic matrix from which the drug is gradually leached, the insoluble plastic 'sponge' being excreted

The first two of the above require some further explanation

1 In these capsules the finely powdered drug is made to adhere to sugar granules by means of a suitable adhesive. The granules are next treated in batches with protective coatings which will delay the release of the drug. Each batch receives a different thickness of coating. The batches are then mixed thoroughly and suitable doses are placed in hard capsules. The batches may be coloured differently to simplify identification and ensure good mixing.

As each capsule contains several hundreds of granules a very good approximation to regular release of drug is obtained. This is due, not only to the differences in the thickness of the coatings, but also to the inevitable differences in the thickness of the coating within any batch. This means that in the batch there is a considerable range in time of release.

Tablets are also made on a similar principle. In this case coated pellets are compressed in a soft matrix to avoid damage to the coatings.

The materials used to protect the granules are of the type used for enteric coating, e.g. synthetic resins, fats and waxes. This means that coated particles are unlikely to be released in the stomach. The sustained release capsules, however, commonly contain untreated granules which will have a more or less immediate effect.

2 Ion exchange resins are insoluble in water and are non toxic. Drugs may be combined with them and the product taken orally with safety. In passing through the body the drug ions are given up by the resin in exchange for ions in the digestive tract. The dose level may thus be maintained for from 10 to 12 hours. The insolubility and ready excretion of the resins make them satisfactory 'carriers' for oral drugs.

Cation exchange resins are used for basic drugs such as dexamphetamine and hyoscamine, and anionic resins are used for acidic drugs such as the barbiturates.

For useful reviews on this subject the student is referred to Lazarus and Cooper (1959), and Carter and Woodford (1961). Blythe (1958) describes methods for the preparation and testing of sustained release products, and further investigations on the testing of such preparations are reported by Souder

and Ellenbogen (1958), Campbell and Theivagt (1958), Vliet (1960) and Royal (1959)

## RECENT ADVANCES IN TABLET COMPRESSION

Until about 1950 most of the investigations into tablet compression were of a practical and empirical nature. Since then, however, a considerable amount of work has been done on some of the problems from a fundamental point of view. In particular, a series of studies by Higuchi and colleagues under the general title, 'The Physics of Tablet Compression,' is of particular interest (1952-1960).

It is not intended here to give more than an outline of this work but the references quoted should be readily available to pharmacy students and referred to when detailed information is required.

The investigations have been of two general types—

- (a) Static
- (b) Dynamic

### (a) STATIC TESTS ON MATERIALS

These tests involve examination of the properties of the materials and of the finished tablets. For example, the flow properties of granules have been investigated by the measurement of the *angle of repose*, Train (1958), Craik (1958). This is the angle formed at the base of a cone formed when the granules fall from an orifice on to a flat surface. This angle is a measure of the inter particulate friction, a small angle, 30 to 40, indicating good flow properties. Factors affecting it include particle size, proportion of fines, the presence and proportion of lubricant and moisture content. As a result of these experiments optimum figures for these factors are deduced.

### *Static Tests on Prepared Tablets*

These involve investigation into the relationship between compression and such data as—

- 1 Specific surface area
- 2 Apparent and true density
- 3 Porosity
- 4 Hardness and mechanical strength
- 5 Disintegration

Specific surface area in a tablet may be measured by a nitrogen adsorption process, Higuchi *et al* (1953). It increases with increasing pressure to a maximum, due to the fracture of particles thus exposing new surfaces, and then declines due to particles being bound together.

Apparent density is the quotient of weight and

geometrical volume. True density is the apparent density minus the enclosed spaces. The true density may be measured by means of a helium densitometer, Higuchi *et al* (1953).

Porosity is expressed as follows—

$$\text{Percentage porosity} = \left( 1 - \frac{\text{apparent density}}{\text{true density}} \right) 100$$

An interesting account of the relationship between compression force and apparent density, porosity, hardness and disintegration is given by Higuchi and colleagues (1953). Using sulphathiazole tablets, they show the increase in apparent density and decrease in porosity with increasing compression, also the increase in hardness and in disintegration time with increasing compression.

Train (1956) investigated the transmission of forces throughout a compacted mass by compressing a powder (Heavy Magnesium Carbonate) in layers, each alternate layer being coloured with ammoniated carmine. The compressions were made with and without lubricant. The resulting compacts were cut longitudinally, and the heights of the layers at various points measured. This showed that compression is not uniform through the mass, areas of greater compression, for example, being at the periphery at the top of the compact. Release of pressure at this part, on the extrusion of the tablet from the die, is considered to be the basic cause of capping.

### (b) DYNAMIC TESTS

Dynamic methods of investigation examine the effects which occur during compression and not on the finished tablets.

These methods were greatly advanced by the use of an instrumented tablet machine, that is, one in which strain and displacement gauges were incorporated, Higuchi *et al* (1954). These make possible the measurement during compression of the following—

- The force exerted by the upper punch.
- The force transmitted to the lower punch.
- The displacement of the upper punch.
- The ejection force.

By using this type of machine it has been possible to estimate the effect of type and quantity of lubricant, the distribution of pressures within the tablet during compression and the energies expended.

These investigations have been carried out with a limited number of medicaments but it is clear that this work is giving a much clearer picture of the problems involved in tablet compression and will clarify problems of formulation.

## REFERENCES

- ANON (1955) The Coating of Tablets by Compression, Machines Available in Great Britain, *Pharm J* 174, 362-365
- BERRY, H (1959) The Measurement of the Rate of Disintegration of Compressed Tablets, *Quart J Pharm* 12, 501
- BERRY, H and RIDOUT, C W (1950) A Study of the Value of Potato Starch and Alginic Acid as Disintegrating Agents, *J Pharm Pharmacol* 2, 619-626
- BLYTHE, R H (1958) The formulation and Evaluation of Sustained Release Products *Drug Standards*, 26, 1-7
- CAMPBELL, D J and THEIVAGT, J G (1958) Determination of Drug Release from Gradual Release Preparations, *Drug Standards* 26, 73-76
- CARTER, S J and WOODFORD, R (1961) Long Acting Oral Medicaments *Pharm Digest* 25, 183-189
- COOPER, B F and BRECHT, E A (1957) Surfactants in Tablets to Improve Disintegration *J Amer Pharm Ass (Sci Edn)*, 46, 520-524
- COOPER, J and PASQUALE, D (1958) The Present Status of Compression Coating, *Pharm J* 181, 397
- CRAIK, D J and MILLER, B F (1958) The Flow Properties of Powders under Humid Conditions, *J Pharm Pharmacol*, 10, 73-79
- \*ELOWE, L N, HIGUCHI, T and BUSSE, L W (1954) The Physics of Tablet Compression VII Compressional Behaviour of Phenacetin and Methacetin Granulations, *J Amer Pharm Ass (Sci Edn)*, 43, 718-721
- EVERS, N (1952) Tests for Tablets, Uniformity of Weight, Disintegration *Pharm J*, 168, 144
- FIRTH, A (1948) Compressed Tablets, a Study in Dimensions *Pharm J*, 161, 383-385
- GWILT, J R (1960) Multilayer Tablets, *Pharm J* 184, 26
- HADGRAFT, J W (1945) Letter about tablet strengths *Pharm J*, 154, 183
- \*HIGUCHI, T, ARNOLD, R D, TUCKER, S J and BUSSE, L W (1952) The Physics of Tablet Compression I A Preliminary Report, *J Amer Pharm Ass (Sci Edn)*, 41, 93-96
- \*HIGUCHI, T, WARSIMHA, R A, BUSSE, L W and SWINTOSKY, J V (1953) The Physics of Tablet Compression II Influence of Degree of Compression on Properties of Tablets, *ibid*, 42, 194-199
- \*HIGUCHI, T, NELSON, E and BUSSE, L W (1954) The Physics of Tablet Compression III The Design and Construction of an Instrumented Tablet Machine, *ibid*, 43, 344-348
- \*HIGUCHI, T, ELOWE, L N and BUSSE, L W (1954) The Physics of Tablet Compression V. Studies on Aspirin, Lactose-aspirin and Sulphadiazine Tablets, *ibid*, 43, 685-689
- HOYLE, H (1946) The Disintegration of Tablets, *Quart J Pharm*, 15, 279
- LAZAREES, J and COOPER, J (1959) Oral Prolonged Action Medicaments their Pharmaceutical Control and Therapeutic Aspects, *J Pharm Pharmacol* II, 257-290
- \*NELSON, E, NAQUI, S M, BUSSE, L W and HIGUCHI, T (1954) The Physics of Tablet Compression IV The Relationship of Ejection and Upper and Lower Punch Forces during Compressional Process Applications of Measurements to Comparison of Tablet Lubricants *J Amer Pharm Ass (Sci Edn)*, 43, 596-602
- \*NELSON, E, BUSSE, L W and HIGUCHI, T (1955) The Physics of Tablet Compression VII Determination of Energy Expenditure in the Tablet Compression Process, *ibid*, 44, 223-225
- \*NELSON, E (1955) The Physics of Tablet Compression VIII Some Preliminary Measurements of Die Wall Pressure During Tablet Compression, *ibid*, 44, 494-497
- PRANCE, H P, STEPHENSON, D and TAYLOR, A (1946) The Disintegration of Compressed Tablets *Quart J Pharm* 15, 286
- ROGERS, A R (1956) Some Statistical Aspects of Quality Control and Standardisation of Tablets, *J Pharm Pharmacol*, 8, 1112
- ROYAL, J, (1959) A Comparison on *In Vitro* Rates of Release of Several Brands of Dextro Amphetamine Sulphate Sustained Release Capsules, *Drug Standards*, 27, 1-6
- SHOTTON, E and GANDERTON, D (1960) The Strength of Compressed Tablets Part I The Measurement of Tablet Strength and its Relation to Compression Forces, *J Pharm Pharmacol*, 12, 87-92
- SHOTTON, E and GANDERTON, D (1960) The Strength of Compressed Tablets Part II The Bonding of Granules During Compression, *ibid*, 12, 93-104
- SMITH, A N (1949) Compressed Tablets Standard Dimensions, *Pharm J* 161, 346 and 381-382
- SMITH, A N (1949) Compressed Tablets Resistance to 'Wear and Tear', *Pharm J*, 182, 194-195, 227-228, 477-478 and 165, 132-133
- SOUDER, J C and ELLENBOGEN, W C (1958) Laboratory Control of Dextro Amphetamine Sulphate Sustained Release Capsules, *Drug Standards*, 26, 77-83

\* These belong to a series under the general heading of The Physics of Tablet Compression

## 9

## Lotions, Liniments and Applications

### LOTIONS

THESE are fluid preparations for application to the skin without friction or for use as washes, e.g. as mouth-washes, gargles, solutions for urethral and vaginal irrigation.

#### Containers for Lotions

Gargles and mouth washes should be dispensed in white fluted bottles. This distinguishes them from medicines for internal use. The labelling should also clearly indicate how they are to be used. They are commonly issued in concentrated form with directions for dilution before use.

If poisonous substances are present the solution should be coloured as an additional distinguishing feature. Amaranth Solution, Sulphan Blue Solution and Compound Tartarazine Solution are used in B.P.C. gargles and mouth-washes.

Eye lotions should also be dispensed in coloured bottles which clearly distinguish them from internal

medicines. Glass stoppers or screw caps with plastic liners are suitable closures. Corks and cork liners are best avoided and in any case should not come into contact with the lotion.

Liniments and lotions should be dispensed in bottles easily distinguishable by touch from those used for internal medicines. Coloured fluted bottles are recommended. Those containing poisons are commonly tinted to further distinguish them (see Exercise 9.2). The labelling should include the words—For external use only.

#### CLASSIFICATION

The exercises included in this chapter are based upon the following classification of lotions—

Class 1 Simple Lotions

Class 2 Eye Lotions

Class 3 Lotions containing Insoluble Substances

#### Class 1. Simple Lotions

Many lotions are simple solutions of one or more substances, which are prepared in a similar manner to Simple Mixtures. The examples which follow

present certain features not already discussed under Mixtures

#### EXERCISE 9.1

##### *Recipe—*

Lotionis Plumbi

3 iv

Signa Super linteum applicandum

The formula is—

Strong Solution of Lead Subacetate 20 ml

Distilled Water, freshly boiled and cooled,  
sufficient to produce 1,000 0 ml

The quantity of Strong Solution of Lead Subacetate is found as follows—  
1,000 oz Dilute Solution is made from 20 ounces Strong Solution

$$\begin{aligned}
 4 \text{ oz Dilute Solution is made from } & \frac{20 \times 4}{1,000} \text{ oz Strong Solution} \\
 & = \frac{20 \times 4 \times 480}{1,000} \text{ minims} \\
 & = 38.4 \text{ minims}
 \end{aligned}$$

This quantity is not measurable, therefore, using the metric system, prepare 120 ml, e.g.

$$\frac{20}{1,000} \times 120 = 2.4 \text{ ml}$$

The distilled water used in preparing this solution must be free from carbon dioxide, in order to prevent precipitation of a basic lead carbonate. Place about 150 ml of distilled water in a flask, attach a Bunsen valve, and boil the water for 10 minutes. Allow to cool before using. From this use the necessary quantity to complete the above preparation.\*

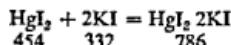
\* Reserve for Exercise 9.11

---

### EXERCISE 9.2

Given Mercuric Iodide and Potassium Iodide, prepare 10 oz of a 1 in 250 solution of the soluble double compound, Mercuric Potassium Iodide  $\text{HgI}_2 \cdot 2\text{KI}$ . Colour suitably.

*Calculation*



35 grains will make 8 oz of a 1 in 100 solution

$$\begin{aligned}
 \frac{35 \times 100 \times 10}{250 \times 8} \text{ grains will make 10 oz of a 1 in 250 solution} \\
 & = 17.5 \text{ gr}
 \end{aligned}$$

$$\text{Mercuric Iodide required} = \frac{454 \times 17.5}{786} \text{ gr} = 10 \text{ gr.}$$

$$\text{Potassium Iodide required} = \frac{332 \times 17.5}{786} \text{ gr} = 7.5 \text{ gr}$$

It is customary to tint solutions of mercuric potassium iodide pink with eosin, and mercuric chloride lotions blue or violet with methylene blue or methyl violet respectively.

The quantity of dye needed is very small, being about 1/25 grain in the above quantity, the object being merely to tint the solutions to make them distinguishable when poured out in bowls for sterilising the skin.

Add the mercuric iodide to the potassium iodide dissolved in a little water (mercuric iodide will dissolve only in a fairly concentrated solution of potassium iodide) dissolve and dilute almost to volume. Add 10 minims of a 0.5 per cent solution of eosin and adjust to volume.

Biniode of Mercury is a synonym (formerly official) for Mercuric Iodide. Thus, if a lotion containing 0.1 per cent of Mercury Biniode is prescribed, a lotion containing this proportion of Mercuric Iodide must be dispensed. It is customary to use an equal quantity of Potassium Iodide to dissolve the Mercuric Iodide, i.e., somewhat in excess of the quantity theoretically required.

## METHODS FOR SATURATED SOLUTIONS

Saturated solutions of definite chemical substances may be prepared by two methods—

(a) By calculation from the known solubility

The method of stating solubilities in the B P is to give the number of parts of solvent (by volume) which will dissolve one part of substance. For example, the B P states that boric acid is soluble in 20 parts of water, meaning that 1 G will dissolve in 20 ml of water.

The volume of such a solution will be more than 20 ml but less than 21 ml, as 1 G of boric acid will occupy less than the volume of 1 ml of water.

In practice, the volume is taken as 21 ml, and a 1 m 21 w/v solution is supplied as a saturated solution, though it is not quite saturated. This is desirable to prevent the solute depositing should the temperature fall.

### EXERCISE 9 3

Prepare 8 oz of a saturated solution of Picric Acid.

The solubility of Picric Acid is known, therefore the calculation is—

The B P states that Picric Acid is soluble in 90 parts of water. Hence, from the above formula the solution to be supplied will be 1 m  $(90 + 1) = 1 \text{ m } 91$ . The calculation is—

35 gr will make 8 oz of a 1 m 100 solution.

$$\frac{35 \times 100 \times 8}{91 \times 8} \text{ gr will make } 8 \text{ oz of a } 1 \text{ m } 91 \text{ solution} = 38.4 \text{ gr} *$$

38.5 gr are weighed.

Follow Method (a) for Saturated Solutions above.

\* If the picric acid is damped, allowance must be made for the added water.

### EXERCISE 9 4

Send 4 oz of a solution of Potassium Permanganate so that one tablespoonful diluted to a quart with water makes a 1 m 4000 solution.

*Calculation*

35 gr will make 8 oz of a 1 m 100 solution.

$$\frac{35 \times 100 \times 40}{4,000 \times 8} \text{ gr will make } 40 \text{ oz of a } 1 \text{ m } 4,000$$

$= 4.375 \text{ gr}$

This quantity must be contained in each tablespoonful of the solution to be sent.

$$4.375 \times 8 = 35 \text{ gr is required for the } 4 \text{ oz.}$$

This and similar preparations may be labelled in one of two ways:

1 If the solution is to be handed to a patient for his use it should be labelled "The Solution—One tablespoonful to be diluted to a quart with water."

2 If the solution is to be held by a doctor or nurse for administration by them it should be labelled "Solution of Potassium Permanganate" "One tablespoonful diluted to a quart makes a 1 m 4000 solution"

Hence the strength of a saturated solution expressed as a w/v solution is 1 m (solubility figure plus 1). The calculation is then identical with the examples given under 'Percentage Calculations,' Exercise 3 3 (p. 23).

The saturated solution is prepared by placing the solute in a beaker or flask, adding as much solvent as solution to be prepared (this allows a little for loss of solvent by evaporation), heating until the solute dissolves, cooling to room temperature or 20°C, and at once adjusting to volume.

(b) By heating the solvent considerably above 20°C, stirring continuously and adding the solute until some remains undissolved, and then cooling to room temperature. The presence of excess of solute prevents the formation of a supersaturated solution. This method is used when the solubility of the solute is not known.

**EXERCISE 9.5**

Send 6 oz of a saturated solution of Thymol containing 2 per cent of Solution of Formaldehyde

Label 'The Mouthwash'

Thymol, in common with menthol and camphor, is slightly soluble in water, imparting its odour and taste. The method of preparing saturated solutions of this kind has already been described on p. 124, *q.v.* First prepare 6 oz of thymol water, using 3 gr of thymol

The calculation for the Solution of Formaldehyde is—

$$\frac{480 \times 6 \times 2}{100} = 57.6 \text{ minims}$$

Dilute 58 minims of Formaldehyde Solution to 6 oz with the thymol water

**Class 2. Eye Lotions**

Eye lotions should be used warm to avoid discomfort and possible irritation of the eye. It is common practice, therefore, to issue eye lotions double strength so that the patient can add warm water to dilute the lotion for use. The lotion is usually applied to the eye by means of an eye-bath. Because of the manner in which eye lotions are used it is unlikely that the product applied to the eye is sterile. Eye lotions are not normally applied to damaged eyes and the same dangers do not exist as in the case

of eye-drops. Nevertheless scrupulous care should be taken in the preparation of eye lotions, and patients should be instructed to ensure that the eye-bath, is thoroughly cleansed and rinsed, with recently boiled water, before use.

If an eye lotion, issued at double strength is to be isotonic with lachrymal secretion in use, any calculation for a quantity of adjusting substance must be taken into account.

**EXERCISE 9.5**

Boric acid 2 per cent

Sodium chloride q.s

Purified Water to 100 per cent

Prepare and send 8 fluid oz, isotonic  
with lachrymal secretion

Label The Eye Lotion To be used with an equal quantity of warm water

*Method*

Eye lotions should be made with freshly boiled and cooled Purified Water. Measure, funnel, container, and any other apparatus to be used should first be well rinsed with some of this water after thorough cleansing. Therefore, boil a suitable excess of Purified Water—about 20 fluid ounces and allow to cool somewhat.

The ingredients are dissolved in some of the water and filtered. Sufficient of the water is passed through the filter to adjust the solution to volume. Filter paper or a sintered glass filter should be used, if the latter, care should be taken to ensure that it is thoroughly washed out after use.

*Calculation*

For the boric acid—

35 gr will make 8 fl. oz of a 1 per cent solution

$35 \times 2 \text{ gr will make } 8 \text{ fl. oz of a } 2 \text{ per cent solution}$

= 70 grains

For the sodium chloride—(See p. 250)

(continued overleaf)

**Exercise 9.5 continued**

The B.P.C. formula is used to calculate adjustment to isotonicity with lachrymal secretion but since the solution is to be diluted with an equal quantity of water for use the figure for the depression of freezing point of tear secretion is doubled. (See also p. 252)

$$\frac{(0.52 \times 2) - (2 \times 0.288)}{0.576} = \frac{0.464}{0.576} = 0.805 \text{ per cent}$$

35 gr will make 8 fl. oz of a 1% solution

$35 \times 0.8 \text{ gr} \text{ will make } 8 \text{ fl. oz of a } 0.8 \text{ per cent} = 28 \text{ grains}$

**EXERCISE 9.6***Recipe—*

Zinci Sulphatis 0.1  
Solutionem Acidi Borici Semisaturatam ad 100.0

Fiat collynum Signetur Pro oculo sinistro Mitte 3 x

*Method*

Follow the general directions given under Exercise 9.5

*Calculation**(a) For the zinc sulphate—*

10 oz of a 0.1 per cent solution is required.

35 gr will make 8 oz of a 1 per cent solution

$$\frac{35 \times 0.1 \times 10}{8} \text{ gr will make } 10 \text{ oz of a } 0.1 \text{ per cent solution} \\ = 4.375 \text{ gr}$$

*(b) For the boric acid—*

Boric acid is soluble in 20 parts of water, consequently, a saturated solution expressed as a w/v solution will be

$$1 \text{ in } (20 + 1) = 1 \text{ in } 21$$

a half-saturated solution will be 1 in 42

10 oz of a 1 in 42 solution is required.

35 gr will make 8 oz of a 1 in 100 solution

$$\frac{35 \times 100 \times 10}{42 \times 8} \text{ gr will make } 10 \text{ oz of a } 1 \text{ in } 42 \text{ solution} \\ = 104.2 \text{ gr Weigh } 104 \text{ gr}$$

Weigh out 5 grains of zinc sulphate ( $= \frac{4}{60} \text{ gr}$ ) and dissolve in 40 drachms of boiled distilled water, measure off 35 drachms of this solution ( $\frac{35}{60} = 4.375 \text{ gr}$ ), add more water to produce about 8 oz, and then add the boric acid. Heat until dissolved, and complete according to the above instructions, using a stoppered bottle

**EXERCISE 9.7***Recipe—*

Solutionis Potassii Permanganatis

(1 in 5,000) 3 viii

Signa Collyrium, cum tanto aquæ calidæ ter in die utendum

*Calculation*

35 gr will make 8 oz of a 1 in 100 solution.

$$\frac{35 \times 100 \times 8}{5,000 \times 8} \text{ gr will make } 8 \text{ oz of a } 1 \text{ in } 5,000 \text{ solution} \\ = \frac{7}{16} \text{ gr}$$

*Method*

Dissolve 1 grain of potassium permanganate in 10 drachms of boiled distilled water, measure off 7 drachms of this solution, and complete as described above. Do not filter, as the filter paper may cause partial reduction of the permanganate. The bottle should have a glass stopper or screw cap with plastic liner—if a cork is used it should be covered with waxed paper to prevent reduction of the permanganate.

It would be simpler in this case to make 250 ml of solution

$$\frac{250}{5,000} = 0.05 \text{ G}$$

dissolve 50 mg in 250 ml and dispense 8 fluid oz

**EXERCISE 9.8***Recipe—*

Zinci Chloridi	ad	gr x
Aquam		5 x

Fiat collyrum. Signa More dicto utendum

*Note* Zinc chloride usually contains some oxychloride, which is insoluble and causes the solution to become opalescent. The clear liquid may be filtered from the oxychloride, using a No. 3 sintered glass filter. Dilute hydrochloric acid must *not* be added to bring about solution of the oxychloride.

Follow the method described in Exercise 9.5

**Class 3 Lotions containing Insoluble Substances**

Insoluble substances in lotions are treated in exactly the same way as in mixtures, e.g. they are suspended only when they are not easily diffusible. Whether certain substances are sufficiently diffusible and of sufficiently low density for a suspending agent to be omitted depends *inter alia*, upon the nature and quality of the substance.

Gummy suspending agents such as tragacanth are not ideal for lotions because of their sticky nature. Substances such as bentonite and aluminium hydroxide gels are sometimes used. For example, 3 per cent of bentonite is used to stabilise Calamine Lotion, and its dispersal and suspending power is improved by the peptizing effect of sodium citrate. 5 per cent of Unemul (see p. 152) a colloidal, hydrated aluminium hydroxide may also be used for a like purpose.

Insoluble substances occurring in lotions are—

Calamine	Zinc Oxide
Zinc Carbonate	Precipitated Sulphur

The first three may or may not need suspending, but as regards sulphur lotions, the sulphur becomes easily diffusible if a surface-active agent such as sodium lauryl sulphate or Extract of Quillaja is added (See Exercise 9.10).

#### METHOD FOR LOTIONS CONTAINING INSOLUBLE SUBSTANCES

**STAGE 1**

Triturate together the insoluble and soluble powders, and make them into a smooth cream with glycerin (if included in the formula) or with the aqueous vehicle.

**STAGE 2**

Add any other ingredients, transfer to a measure, rinse the mortars with more aqueous vehicle, add to the measure, and adjust to volume.

**EXERCISE 9.9***Recipe—*

Calamine	15.0%	Sodium Citrate	0.5%
Zinc Oxide	5.0%	Liquefied Phenol	0.5%
Bentonite	3.0%	Glycerin	5.0%

Purified water to 100.0%

Prepare 4 fluid ounces

Label To be used often

(continued overleaf)

## Exercise 9.9 continued

It will be convenient to prepare 120 ml, i.e.—

Calamine	18.0 G	Sodium Citrate	0.6 G
Zinc Oxide	6.0 G	Liquefied Phenol	0.6 ml
Bentonite	3.6 G	Glycerin	6.0 ml

Purified Water to 120.0 ml

Dissolve the Sodium Citrate in about 100 ml of Purified Water. Thoroughly triturate the insoluble powders and triturate with the Sodium Citrate solution. Add the Glycerin and Liquefied Phenol and adjust to volume with Purified Water.

## EXERCISE 9.10

## Recipe—

Sulphuris Praecipitati	5.0 G
Glycerini	5.0 ml
Alcoholis	15.0 ml
Aquam Roseæ	ad 100.0 ml

Misce fiat lotion Applicetur omni nocte capiti.

Triturate the sulphur with the glycerin, add 1 ml of Extract of Quillaja, the alcohol, and dilute to volume with the Rose Water (0.25 G of sodium lauryl sulphate may be used in place of Extract of Quillaja)

Precipitation in lotions is usually intentional, the insoluble substance formed by reaction being often the important constituent of the lotion.

Admixture of *dilute* solutions of reacting substances yields a finer precipitate than admixture of strong solutions, hence, where possible, the reacting

substances should be separately dissolved in about half the volume of aqueous vehicle available, and the two portions then mixed. Prepared in this manner the precipitate is usually diffusible, and therefore no suspending agent is added.

## EXERCISE 9.11

## Recipe—

Tincturæ Opii	m. xx
Lotionem Plumbi	ad 5 i

Fiat lotion Mitte 5 iv Signa More dicto utenda.

This lotion is similar to Lead and Opium Lotion. B P C. 1959

Add the tincture to about 3½ fl. oz of Lead Lotion and adjust to volume with a further quantity of lotion. The precipitate, which is readily diffusible, consists principally of lead meconate, resulting from the interaction of the basic lead acetate in the Lead Lotion and the alkaloidal meconates in the Tincture of Opium.

## EXERCISE 9.12

## Recipe—

Zinci Sulphatus	5 J
Potassæ Sulphuratae	5 J
Aquam	ad 5 iv

Fiat lotion Signa Nocte adhibenda.

Note Sulphurated Potash is a mixture of potassium sulphides and other potassium compounds, and should be reasonably fresh, otherwise it is fairly insoluble. Zinc sulphate interacts with the former, forming insoluble zinc sulphide, which is diffusible.

Dissolve each ingredient in sufficient water to produce 2 oz, and mix.

## LINIMENTS

Liniments are liquid or semi liquid preparations typical liniments are included in the following intended for application to the skin Many of exercises—them are emulsions or simple solutions, and some

**EXERCISE 9.13**

Prepare 2 oz of Camphorated Oil

Label The Liniment

Prepare 60 grammes from the official formula Weigh out the arachis oil in a dry wide-mouthed bottle fitted with a perfectly fitting stopper or cork, add the camphor and tie down the stopper Place in a cold water-bath, and heat until the camphor dissolves (*circa* 75°C) Shake occasionally but do not remove the stopper until the solution has cooled to room temperature Measure the required volume into a dry bottle, and complete as usual

**EXERCISE 9.14**

*Recipe—*

Glacial Acetic Acid

10 ml

Liniment of Camphor

45 ml

Turpentine Oil

to 100 ml

Label Turpentine and Acetic Acid Liniment

Use the Liniment of Camphor prepared for Exercise 9.13, the measuring cylinders and bottle must be quite dry

**EXERCISE 9.15**

*Recipe—*

Mentholis

ana 5 J

Camphoræ

Thymolis

Fiat linimentum Signa Ope penicilli parti affectæ applicandum

The above ingredients form an oily liquid when mixed together Therefore powder all the ingredients together in a dry mortar until a liquid is formed, then transfer to a dry bottle

### B.P., B.P.C. AND OTHER LINIMENTS

**LINIMENT OF ACONITE**
**LINIMENT OF BELLADONNA**

These are prepared by a process of extraction, described in *Tutorial Pharmacy*

**LINIMENT OF ACONITE, BELLADONNA AND CHLOROFORM (Sjn A B C Liniment)**

Aconite Liniment

Belladonna Liniment

Camphor Liniment

Chloroform

The liniment is prepared by vigorous shaking

**LINIMENT OF CAMPHOR, B.P.**

(*Sjn* Camphorated Oil)

Camphor

Arachis Oil

The preparation is described above

**LINIMENT OF CAMPHOR, AMMONIATED**

Camphor

Oil of Lavender

Strong Solution of Ammonia

Alcohol (90%)

The first two ingredients are dissolved in most of the alcohol, and the Strong Solution of Ammonia

then added in portions, shaking after each addition. The ammonia solution, being aqueous, dilutes the alcohol around the zone of entry, and this precipitates a portion of the camphor, which redissolves upon shaking. Sufficient alcohol is then added to produce the required volume.

#### LINIMENT OF METHYL SALICYLATE

Methyl Salicylate

Arachis Oil or Cotton Seed Oil

#### WHITE LINIMENT

Oleic Acid

Dilute Ammonia Solution

Ammonium Chloride

Turpentine Oil

Water

Mix the dilute ammonia solution with an equal volume of warm water and place in a bottle. Add the mixed acid and oil gradually while shaking. Finally add the ammonium chloride in the remaining water.

#### LINIMENT OF TURPENTINE B.P. 1963

The preparation of this is described in Exercise 512, p. 64.

#### LINIMENT OF SOAP

Camphor	40 G
Oleic Acid	40 G
Alcohol, 90 per cent	700 ml

Potassium Hydroxide Solution	a sufficient quantity
Rosemary Oil	15 ml
Purified Water	to 1000 ml

Dissolve the oleic acid in 500 ml of the alcohol and add potassium hydroxide solution until one drop of the solution diluted with ten drops of carbon dioxide-free water gives a full blue with one drop of bromothymol blue and a full yellow with one drop of thymol blue. The former colour indicates a range of pH 7.6 and higher, the latter indicates a range of pH 8 and lower. Thus, the adjusted pH lies between 7.6 and 8. This requires about 140 ml of potassium hydroxide solution.

Dissolve the camphor and the rosemary oil in the remainder of the alcohol and mix the two solutions. Add sufficient purified water to produce 1,000 ml and allow the product after mixing to stand for at least seven days, and filter it.

### B.P. AND B.P.C. LOTIONS

*Calamine Lotion* is the only lotion in the Pharmacopoeia. It has the formula given in Exercise 99. B.P.C. lotions are mostly aqueous solutions.

*Lotion of Copper and Zinc Sulphate Solvent—Camphor Water*

*Lotion of Lead* Exercise 91

*Lotion of Lead, Evaporating* Contains 12.5 per cent of 95 per cent Alcohol

*Lotion of Zinc Sulphate* An aqueous solution tinted with Amaranth Solution

#### LOTION OF SALICYLIC ACID AND MERCURIC CHLORIDE

Salicylic Acid

Mercuric Chloride

Acetone

Castor Oil

Alcohol (95 per cent)

Dissolve the mercuric chloride and the salicylic acid in 500 ml of the alcohol and add the castor oil, acetone and sufficient of the alcohol to produce the required volume.

In addition to the words 'For external use only' the lotion should be labelled 'Inflammable, keep away from a naked flame'.

#### LOTION OF CALAMINE, OILY

Synonym *Linimentum Calamineæ*

Calamine

Wool Fat

Arachis Oil

Oleic Acid

Calcium Hydroxide Solution

Melt together the wood fat, arachis oil and oleic acid and triturate the calamine with the mixture. Transfer to a suitable container, add the calcium hydroxide solution and shake vigorously to emulsify. This is an example of a fluid 'Lime Cream' (see Exercise 1010) in which a water-in-oil emulsion is formed by the emulsifying property of calcium olate. Wool fat also helps to stabilise the emulsion.

#### LOTION OF HYDROCORTISONE

Self-emulsifying Monostearin

Hydrocortisone, in ultra-fine powder

Chlorocresol

Glycerin

Purified Water

Dissolve the chlorocresol in most of the purified

water by gentle heating, add the self emulsifying monostearin and disperse it by heating to 60°C with stirring. To the warm dispersion add the glycerin with which the hydrocortisone has been triturated. Stir until cold and add sufficient purified water to produce the required weight.

## APPLICATIONS

### B.P. AND B.P.C. APPLICATIONS

Applications are similar to lotions in being fluid or semi fluid preparations. They may be solutions, suspensions or emulsions. Those of the B.P. and B.P.C. are emulsions or suspensions and most of them are used as antiparasitic preparations.

#### APPLICATION OF BENZYL BENZOATE B.P.

Benzyl Benzoate  
Emulsifying Wax  
Purified Water

Melt the Emulsifying Wax and add the Benzyl Benzoate. Mix and add the mixture to about half of the warmed Purified Water. Stir until a good emulsion is formed. Gradually stir in the remainder of the Purified Water.

#### APPLICATION OF DICOPHANE

Synonym DDT Application

Emulsifying Wax  
Dicophane  
Xylene, of commerce  
Citronella Oil  
Water

#### APPLICATION OF GAMMA BENZENE HEXACHLORIDE

Emulsifying Wax  
Gamma Benzene Hexachloride  
Xylene of commerce  
Lavender Oil  
Water

In each of the above cases dissolve the active ingredient with the essential oil in the Xylene and mix with the Emulsifying Wax, melted at a low temperature. Add the warm mixture to about half of the water, previously warmed to about the same temperature and stir to emulsify. Add sufficient water to produce the required volume.

#### APPLICATION OF CALAMINE, COMPOUND

This is a suspension of Calamine and Zinc Oxide in a vehicle consisting of Liquid Paraffin, Soft Yellow Paraffin Wool Fat and Zinc Stearate.

#### APPLICATION OF SALICYLIC ACID AND SULPHUR

This is a suspension of the two finely sifted substances in Aqueous Cream.

### REVISION EXERCISES 1 Recipe—

Acidi Salicylici	gr xx
Resorcinolis	gr xx
Olei Olivæ	3 ss
Aquam	ad 3 iv

Fiat lotio pro capillis Signa Omni nocte utenda

### 2 Recipe—

Sodii Bicarbonatis	2 0
Boracis	2 0
Acidi Carbolici	0 5
Sacchari	4 0
Aquam	ad 1000

Fiat collunarium Mitte 3 viii Signa Cum duplo aquæ calidæ nocte manequ utendum

(continued overleaf)

*Revision Exercises continued*3 *Mitte*—

Glycerini Thymolis Compositi . . . . . 3 vi  
 Signa Collutorium Cochleare magnum e semicyatho magno aquæ  
 calidæ quotidie utendum

4 *Recipe*—

Tincturæ Myrrhæ	3 ij
Sodii Baboratis	3 i
Aquaæ Rosæ	3 i
Aquam	ad 3 ii

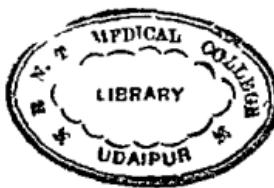
Fiat gargarisma Signa Cum tanto aquæ calidæ

5 *Recipe*—

Zinci Oxidi	3 ii
Ichthammolis	3 i
Liquoris Plumbi Subacetatis Fortis	3 i
Glycerini	3 ii
Aquam	ad 3 iii

Fiat lotio Signa Bis in die applicanda

---



## Ointments, Creams, Pastes and Jellies

### OINTMENTS AND CREAMS

#### *Definition*

Ointments are semi-solid preparations for application to the skin, and usually contain a medicament. The bases are animal, vegetable or mineral fats and oils or synthetic materials. Medicaments may be in solution, suspension or emulsified in the base.

Ointments are used for emollient, protective or other surface effect or they may contain medicaments which are intended to be absorbed systematically. The choice of base in each case is important and is considered at some length later in this chapter.

The term 'water miscible ointment' is sometimes applied to ointments containing an oil-in-water emulsifying agent, e.g. Emulsifying Ointment B.P. or to ointments whose bases consist of mixtures of the water soluble polyethylene glycols, e.g. Macrogol Ointment B.P.C. Such bases mix readily with skin exudates and are readily washed from the skin.

A miscellaneous group of preparations is included in this chapter under the headings of Pastes and Jellies. Some of these are merely ointments containing rather a large proportion of powder, e.g. Lassar's Paste.

Dermatological creams are semi solid emulsions of either oil in-water or water in oil types. Official examples of these are Aqueous Cream and Oily Cream. The various emulsifying agents used in the preparation of creams may involve incompatibility and these are mentioned later in individual cases. It must also be remembered that certain medicaments are unstable in the presence of water, and that emulsion systems are liable to instability.

The term 'cerate' was formerly applied to certain ointments which contained a high proportion of wax (cera) and which were, therefore, harder than normal ointments.

#### GENERAL NOTES ON PREPARATION

##### *Order of Melting with Mixed Bases*

In the past it was considered that the correct procedure, when an ointment consisted of several ingredients of different melting points, was to melt the substance of highest melting point first and add the others in descending order of melting point. This was thought to be quicker and to employ less heat. Price and Osborne (1958), however, state that *this is not the most satisfactory method and that, if the materials are mixed and melted together, the maximum temperature reached and the time taken are less. It is suggested that this is due to the solvent action of the lower melting point substances on those with higher melting points.*

The time of melting is also shortened by shredding waxy components and stirring the ingredients while melting.

Such ointments should, therefore, be made by melting the ingredients together simultaneously, but the student should attempt to verify the above claim by preparing an ointment of this type, e.g. Paraffin Ointment, by both methods, carefully noting the maximum temperature and time taken in each case.

##### *Modification of Base for Rectal Ointments*

To render these ointments sufficiently soft to pass through the fine holes of a rectal nozzle without bursting the tube, it is usual to replace 25 per cent of the base with oil. For replacing Simple Ointment or Lard, arachis or olive oil is used. When the ointment is made with Soft Paraffin, the portion is replaced with liquid paraffin. Ointments prepared with a base of Oily Cream are sufficiently soft for rectal use, and the above modification of base is unnecessary.

### Avoidance of Contact with Metal

Certain substances interact with steel, and in preparing these in ointments it is essential to use a bone or vulcanite spatula. Among these substances are—

Soluble mercuric salts, e.g. mercuric chloride and mercuric nitrate (The insoluble mercuric salts, e.g. mercuric ammonium chloride, mercuric oxides, and the mercurous salts, do not interact.)

Tannic acid, and substances containing it, e.g. galls, Salicylic acid, benzoic acid

Iodine

Bleaching powder, and other hypochlorites

Stainless steel (chromium nickel steel or chromium nickel-molybdenum steel) react with bleaching powder and other hypochlorites, iodine, mercuric chloride, and mercurous chloride (in the presence of moisture), but may be used with benzoic acid, boric acid, oleic acid, salicylic acid, phenol, and tannic acid and substances containing it, without fear of reaction.

### GENERAL NOTES ON PACKING AND LABELLING

Ointments are usually dispensed in pots or collapsible tubes, or in greaseproof boxes. If the pot is fitted with a metal cap, this should be lined with a disc of waxed paper to prevent possible interaction with the medicament. Metal containers, e.g. tins, are not suitable for ointments containing Oily Cream as the base, or for ointments containing a large proportion of aqueous liquid, because the water present leads to corrosion of the metal. Further, contact with metal appears to encourage separation of water from these ointments.

### Collapsible Tubes

These provide a hygienic form of packing, and reduce the possibility of the ointment becoming rancid. They should be made of pure tin, as composition metal is liable to be attacked by certain medicaments.

When the melted ointment does not separate upon cooling, it may be poured directly into the tube, and allowed to cool there. For other ointments the procedure is as follows—

#### Class 1. Ointments

Many of the fatty substances used as bases for ointments, e.g. soft paraffin, are manufactured on a large scale, but there are numerous admixtures which are usually prepared in the pharmacy. Bearing in mind the comments made previously, these ointments should be made by mixing the ingredients, first

Place the ointment in the centre of a sheet of waxed paper about 6 in. x 8 in., fold over along one edge as if wrapping a powder. Holding down the two edges together on the bench, lay a spatula parallel with the fold, and bring it towards the ointment—thus spreading the mass into a cylindrical form. When this has been spread to a diameter slightly less than that of the tube, roll it over the remaining flat portion of the paper, forming a paper tube with the ointment evenly enclosed. Place it in the collapsible tube, fold over the protruding end of the paper until it is level with the end of the tube, and then gently withdraw by repeatedly turning over a small fold.

To seal collapsible tubes, flatten them slightly towards the open end, using a spatula. Then press the spatula across the open end, closing about a quarter of an inch. Holding this firmly, bring the tube gently round to the upright position. Turn down the flap, and make another fold, tighten with pliers, or fix a clip supplied for this purpose.

### Eye Ointments

Eye ointments should be sterile and supplied in small sterile tubes with a fine nozzle for ease of application. Care should be taken to ensure that the tubes are thoroughly clean and free from metallic particles.

### Labelling

Gummed labels can be made to adhere to collapsible tubes by first painting the metal surface to the required extent with Compound Benzoin Tincture, and allowing it to dry. If the edges of the label will not meet, cut a piece of white demy long enough to encircle the tube, and affix this first, then apply the label. It is usual to pack tubes in a cardboard box, suitably labelled.

### CLASSIFICATION

The following classification will serve as a basis for learning the methods of preparing ointments—

*Class 1. Ointments prepared by Fusion*

*Class 2 Ointment Emulsions*

*Class 3 Ointments prepared by Trituration*

*Class 4 Miscellaneous Ointments and Creams*

#### Prepared by Fusion

subdividing any waxy components, and melting together over a water bath. When melted the product should be stirred until cold, avoiding localized cooling which can occur by using a cold spatula for stirring or by transferring to a cold container before the ointment has set. If the ointment is granular, due

to the separation of higher melting point ingredients, it should be remelted and again stirred until cold.

Stock bases of this type containing no insoluble ingredients can be made successfully by transferring to a warm jar when melted, covering and allowing to stand without stirring until set. The more important admixtures are described in Exercises 10 1, 10 2, 10 3 and 10 4.

Simple Ointment is the base of Ammoniated Mercury Ointment B P, Sulphur Ointment B P,

Zinc Ointment B P and Capsicum Ointment B P C

In addition to the ointment bases just described, many medicated ointments are prepared by fusion, especially those containing medicaments which are soluble in the base. In the large scale production of ointments insoluble medicaments are frequently incorporated by stirring them into the melted base and the resulting ointment ground smooth by passage through an ointment mill.

#### EXERCISE 10 1

Prepare 60 grammes of Ointment of Wool Alcohols B P.

Melt the ingredients in a dish over a water-bath, following the directions given above, i.e. shred or cut in small pieces the Wool Alcohols and Hard Paraffin, mix with the Soft Paraffin and Liquid Paraffin and heat in an evaporating basin over a water-bath until melted. The mixture should be stirred to expedite melting and finally stirred until cold. Reserve the ointment for subsequent exercises.

The Wool Alcohols present in this ointment base is a powerful emulsifying agent of the *water in oil* type. The ointment is thus able to take up large quantities of aqueous or hydro alcoholic liquids, and advantage is taken of this property in its use as a base for some of the official ointments.

#### EXERCISE 10.2

Cholestrol	3 G
Stearyl Alcohol	3 G
White Beeswax	8 G
Soft White Paraffin	86 G

The above formula is included in the *U S P XVI* under the title Hydrophilic Petrolatum.

It is prepared in the same way as Wool Alcohols Ointment and is intended for similar purposes.

#### EXERCISE 10 3

Prepare 30 grammes of Emulsifying Ointment B P

This ointment contains 30 per cent of Emulsifying Wax in White Soft Paraffin and Liquid Paraffin. It is made in the same manner as Wool Alcohols Ointment. It differs from the latter ointment in that, on admixture with water, it forms an *oil in water* emulsion. It is used in the preparation of Aqueous Cream and the above quantity should be reserved for this purpose.

#### EXERCISE 10 4

Prepare 60 grammes of Simple Ointment

This ointment will be required later for a white ointment, therefore use white soft paraffin. Make six hundredths of the pharmacoparial quantity, and, in carrying out the official directions, note that it will be preferable to follow the directions for melting given previously. Examine the melted material critically, and if particles of foreign matter are visible, decant or strain through muslin. As mentioned above it is essential, when uniting components of different melting points, to stir constantly during cooling, thereby ensuring a homogeneous product.

**Preparation of Medicated Ointments by Fusion**

In preparing medicated ointments by fusion the following stages should be observed—

**STAGE 1**

Finely powder any *insoluble* solid medicament if necessary, sift through a No. 60 sieve, and weigh out the required amount

**STAGE 2**

Melt the base, or its components, at as low a temperature as possible, add soluble medicaments, and stir until dissolved. Then add the sifted insoluble medicament, and liquids, if any, and stir until cold.

**Particular Substances****LIST A**

*Substances, commonly prescribed in ointments which are soluble in the base*

Camphor	Naphthol
Chloral Hydrate	* Phenol
Menthol	Thymol

*Note* Any pair of these substances forms an oily liquid when triturated together. Hence, an ointment containing two of these with a soft base is prepared by trituration, the resultant oily liquid being simply mixed with the base (Exercise 105).

**EXERCISE 105**

Mitte Unguenti Methylis Salicylatis Compositi B.P.C. 3*ii*

Signa Nonen Proprium

Follow the direction given in the Codex using as low a temperature as possible to avoid loss of the volatile ingredients

Label Compound Methyl Salicylate Ointment

**Class 2. Ointment Emulsions**

Fluid emulsions must contain an emulsifying agent if they are to remain stable for more than a short period. As already mentioned, a temporary emulsion of castor oil may be formed by shaking it vigorously with water, but separation quickly ensues. This temporary emulsion may be called a mechanical emulsion. It is evident that if a non-fatty liquid (*i.e.* one immiscible with fats) is distributed throughout a solid fat by trituration, there will be formed an emulsion which cannot readily separate into its components because one phase (*the fat*) is solid, and prevents coalescence of the distributed globules of non-fatty liquids. It is, therefore, possible to prepare stable mechanical emulsions with fats—the ointment prepared in Exercise 1025 being an example.

True emulsions in solid or semi solid form, e.g. ointments and creams, may be grouped according to the emulgents present, the principal groups being—

**Wool Fat Emulsions****Wool Alcohols Emulsions****Beeswax Emulsions****Soap Emulsions****Synthetic Wax Emulsions**

Each of these is discussed below

**Wool Fat Emulsions**

Wool fat can emulsify about half its own weight of water, when mixed with fats, e.g. soft paraffin, it can emulsify several times its own weight of an aqueous liquid. The resultant emulsions are of the *water-in-oil* type.

Wool fat is too sticky for use alone, and is usually mixed with hydrocarbons, as in the official eye-ointment base, and in Simple Ointment. These products are therefore able to take up an appreciable proportion of aqueous or hydroalcoholic liquids, forming true emulsions, thus B.P. and B.P.C. alkaloidal eye ointments are prepared by incorporating an aqueous solution of alkaloidal salt with the eye ointment base, and these ointments are true emulsions.

Lanolin, or hydrous wool fat is the simplest representative of this group, and Exercise 106 the method of preparation.

\* Phenol is very soluble in natural oils and fats (commonly up to 20%) but is much less soluble in soft or liquid paraffin—2-3 per cent being considered as the maximum which should be included in a purely hydrocarbon base.

**EXERCISE 10 6**

Prepare 30 grammes of Hydrous Wool Fat B P

Follow the pharmacopoeial directions. The wool fat may be melted by placing it in a hot mortar. After addition of the Purified Water, the product should be vigorously triturated until an almost white product is formed.

**Wool Alcohols Emulsions**

Wool Alcohols is the emulsifying principle of Wool Fat. It contains cholesterol, lanosterol and agnosterol and other alcohols.

Its use in the preparation of Wool Alcohols Ointment has already been mentioned, this ointment base is not itself an emulsion, but it is used for the preparation of many ointment emulsions including Oily Cream which forms the subject of Exercises 10 7 and 10 8.

**Beeswax Emulsions**

A number of the higher alcohols (e.g. cetyl alcohol, stearyl alcohol) form emulsions of the water in oil type. Beeswax consists principally of methyl palmitate, i.e. an ester of one of the higher alcohols and its emulsifying properties are doubtless due to this constituent. Although beeswax is not a very good emulsifying agent it is a valuable stabilizing ingredient in many emulsion creams e.g. Exercises 10 12 and 10 9. The emulsions formed are of the water in oil type.

**Soap Emulsions**

Soap is rarely used *per se* as an emulsifying agent in preparing ointment emulsions, but is formed in the process of making the latter. Most vegetable and animal fats and oils contain a small proportion of free fat acid, which combines with alkaline substances (e.g. borax, ammonia, triethanolamine) to form a soap, the latter emulsifies the remainder of the fatty ingredients. The emulsions formed are of the oil in water type. This type is preferable for 'cold creams', because the water, being in the continuous phase, can evaporate freely from a thin film, and in evaporating, withdraws heat from the skin, thus producing a cooling action. The fat remaining on the skin, after more or less complete evaporation of the water, is absorbed slowly—rapid absorption is not the objective, because the fat is included for its emollient action on the superficial layer of the skin.

The inclusion of a natural fat or oil, or a fatty acid is a *sine qua non* for cold creams of this type—the paraffins (hard, soft, or liquid) do not contain

**EXERCISE 10 7**

Prepare 60 grammes of Oily Cream

Melt the Wool Alcohols Ointment prepared in Exercise 10 1 and transfer to a warm mortar. Gradually add the Purified Water, previously warmed to a temperature of about 50°C, stirring constantly. Continue to triturate until the ointment is quite cool. The product should be almost white and quite homogeneous in texture. Although not officially directed the use of warm water in the preparation of this ointment is important; cold water may lead to the formation of small waxy masses in the ointment, with consequent lack of homogeneity.

**EXERCISE 10 8**

Tannic Acid	2 parts
Distilled Water	4 parts
Ointment of Wool Alcohols	4 parts

Label Astringent Ointment'

Melt the Wool Alcohols Ointment and transfer to a warm mortar. Dissolve the Tannic Acid in the Distilled Water, previously warmed to a temperature of about 50°C. Gradually add the solution to the melted base, triturating constantly. Continue stirring until the ointment is cold. It will be observed from the method of preparation that the medicament is present in the disperse phase of the finished emulsion.

free fat acid, and thus they cannot react with the borax to form a soap. A cold cream containing a high proportion of hydrocarbon fats can be prepared, but it is essential to include either a small proportion of a natural fat or oil, or a fatty acid, e.g. oleic acid.

Sodium, potassium, ammonium and triethanolamine soaps are incompatible with mineral acids, which liberate the free fatty acids. They are also incompatible with di- or trivalent metallic ions. Calcium and magnesium salts, for example, cause phase inversion because they form calcium and magnesium soaps. High concentrations of electrolytes have the effect of salting out the soaps. Soap emulsions may also be criticised on dermatological grounds because of their alkalinity.

#### GENERAL METHOD OF PREPARATION

The fatty components are melted at as low a temperature as possible, the alkaline substance together with the aqueous liquid is raised to the same temperature, and the two portions mixed and stirred vigorously until the product sets. It is most important to heat the aqueous alkaline liquid to about the same temperature as the fats—if added cold, the aqueous liquid would cause rapid congelation of the high melting point fats or waxes, and the product would be lumpy.

Classical examples of cold creams were formulated with beeswax and borax. Hydrous Ointment B.P. 1932 (Exercise 10.9) is a typical example and will serve to illustrate preparation of the sodium soap type of cold cream. It should however be noted that this preparation is an oil in water emulsion only during the short period immediately following addition of the borax solution to the fats, during cooling, the emulsion reverts to the water in-oil type.

Soaps of divalent elements such as calcium and zinc form water in oil emulsions and these are

occasionally used in the preparation of dermatological creams. The so called 'Lime Creams' are prepared with a calcium soap prepared from a solution of calcium hydroxide and a suitable fatty acid such as oleic acid. Exercise 10.10 is an official example in which a small proportion of wool fat assists in stabilising the water in oil emulsion.

Such creams are not very stable when prescribed with certain medicaments e.g. Ichthammol. The wool fat helps and it is usual in formulating such creams to increase the wool fat content to ensure stability, as in the case of Zinc and Ichthammol Cream B.P.C.

#### Synthetic Wax Emulsions

The emulsifying agents used in the preparation of ointment emulsions and emulsion creams are commonly prepared as emulsifying waxes. These are seldom single chemical substances and generally consist of two compounds which combine to form a stable complex. The great stability of emulsions prepared with these complex agents has been explained by Schulman and Cockbain (1940) and an account of this is given in *Tutorial Pharmacy*. Emulsifying Wax B.P. is an example of such an emulsifying agent and consists of Sodium Lauryl Sulphate and Cetostearyl Alcohol.

The emulsifying waxes are used in formulating emulsifying ointments. Such ointments are capable of emulsification with water to form emulsions, usually of the oil in-water type.

This is well exemplified by the following—  
Emulsifying Wax B.P. contains—

Sodium Lauryl Sulphate	10 per cent
Cetostearyl Alcohol	90 per cent

Emulsifying Ointment B.P. contains—

Emulsifying Wax	30 per cent
Liquid Paraffin	20 per cent
White Soft Paraffin	50 per cent

#### EXERCISE 10.9

White beeswax	18 G
Borax	1 G
Almond Oil	61 G
Rose Oil	0.1 ml
Rose Water	20.0 ml

Label The Cold Cream

#### EXERCISE 10.10

Prepare 50 grammes of Zinc Cream B.P.

Label The Zinc Cream

Follow the official directions

**Aqueous Cream contains—**

Emulsifying Ointment	30 per cent
Chlorocresol	0.1 per cent
Purified Water	69.9 per cent

The following list includes the substances in common use for the preparation of water miscible ointments and creams

**Non-ionic**

Glycerol esters of fatty acids

Glycol esters of fatty acids

Sorbitan esters of fatty acids

Polyethylene glycol esters of fatty acids

Polyethylene glycol ethers of fatty alcohols

Polyethylene glycol derivatives of sorbitan esters of fatty acids

**Anionic**

Soaps or alcohol sulphates combined with fatty alcohols

**Cationic**

Quaternary ammonium compounds combined with fatty alcohols

**Type of Emulsion—Oil-in-water or Water-in-oil**

Some of the above agents produce oil in water and some water-in-oil emulsions. The following brief explanation will help in an understanding of this property.

In non-ionic emulsifying agents the molecule consists of a hydrophilic (water soluble) part and a lipophilic (oil soluble) part. Hydroxyl (OH) groups and ethylene oxide ( $\text{CH}_2\text{OCH}_2$ ) groups confer hydrophilic properties on the compound, the long chain fatty acid group (usually 12 to 18 carbon atoms) confers lipophilic properties. The molecule becomes adsorbed at the oil/water interface forming a stable emulsifying film with the oil-soluble groups directed towards the oil and the water-soluble groups directed towards the water. If the effect of the water-soluble part is greater than that of the oil soluble part the emulsion will be of the oil in-water type, if the oil-soluble part is dominant a water in-oil emulsion results.

Thus, the glycol, glycerol and sorbitan esters of the fatty acids produce water in-oil emulsions, since the lipophilic fatty acid radicle is more powerful than the hydrophilic glycol, glycerol or sorbitan radicles. Note carefully, however, that the addition of monovalent soaps to these compounds reverses the type of emulsion as in the case of Self Emulsifying Monostearin B P C. The polyethylene glycol com-

pounds mentioned in the above list, provided the number of oxyethylene groups in the molecule is more than ten, all produce oil-in-water emulsions since the oxyethylene groups confer strong water-soluble properties on the compounds.

In the anionic and cationic agents mentioned, combination occurs between the water-soluble soaps, alcohol sulphates or quaternary compounds on the one hand, the the oil-soluble fatty alcohols on the other. In these cases the water-soluble components are dominant and the emulsions formed are oil in-water.

**Non ionic****GLYCOL AND GLYCERYL ESTERS**

Emulsifying agents in this group include a number of wax-like solids which have been developed for the preparation of water-miscible ointment bases, cold and vanishing creams. They are non-ionic substances, being esters of certain of the higher fatty acids. Of the many which have been introduced as emulsifying agents the following are examples—

Glyceryl monostearate

Glyceryl mono-oleate

Diethylene glycol stearate

Propylene glycol stearate

In the pure form they are lyophilic substances, producing water-in-oil emulsions, but as supplied in commerce they usually contain a trace of soap or other oil in-water emulsifying agent such as sodium lauryl sulphate, which confers on them oil in-water emulsifying properties.

Self-emulsifying Monostearin B P C is an example of this type consisting of the mono-, di- and tri-glyceryl esters of stearic and palmitic acids, with small quantities of the corresponding esters of oleic and other fatty acids, together with free fatty acids and glycerin. Such a mixture would produce a water in oil emulsion but the preparation also contains traces of sodium, potassium or triethanolamine oleates and stearates. These soaps convert the preparation to an oil in-water promoting emulsifying wax.

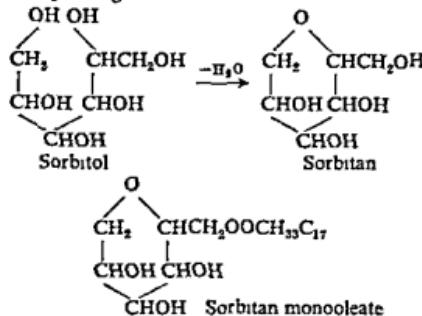
Self emulsifying Monostearin B P C is a white or cream coloured waxy solid with a faintly fatty odour and taste. It melts at 54° to 57°C. It is insoluble in water but may be dispersed finely in hot water. It is soluble in hot vegetable oils. Since soap is an ingredient it is an anionic preparation and incompatible with mineral acids, calcium salts, high concentrations of electrolytes and zinc oxide. The glyceryl monostearate, however, has a marked stabilising influence and the emulsions are, therefore,

more resistant to acids and calcium salts than soap emulsions. They are also less alkaline, a point of dermatological importance.

From 5 to 20 per cent may be used in the formulation of creams, Exercise 10.11 being an example.

#### SORBITAN ESTERS

Sorbitans are derived from sorbitol by loss of a molecule of water. One form of sorbitan and the corresponding oleic ester is shown below:



Sorbitan esters of fatty acids notably lauric, palmitic, stearic and oleic are water in-oil producing emulsifying agents and may be used in forming stable creams. Exercise 10.12 is an example of one of these creams which is of a fine texture.

Such creams are stable in the presence of considerable amounts of medicaments. Spalton (see References) states that they are stable with ich thammol, benzoic acid, salicylic acid, sulphur, ammoniated mercury, coal tar and resorcinol.

A series of sorbitan esters are marketed in the U.S.A. under the name *Span*. A number is used to identify the individual member. *Span* 80, for example, is sorbitan mono-oleate. These are used commonly as stabilisers with one or other of another series of compounds known as *Tweens* in the formulation of oil in water creams. The *Tweens* are polyoxyethylene derivatives of the *Spans* and are described later in this chapter.

#### POLYETHYLENE GLYCOL DERIVATIVES

Polyethylene glycols are prepared by the polymerisation of ethylene oxide and have the following general formula—



They are water soluble and form a series of compounds ranging from liquids (molecular weights 200 to 700) to wax like solids (molecular weights 1,000 and over) depending on the length of the chain. Mixtures of these are used as water soluble ointment bases, e.g., Macrogol Ointment B.P.C. and are described later (p. 161). Their particular interest here is that they form valuable emulsifying agents.

#### EXERCISE 10.11

##### Self-emulsifying Monostearin

8.0 G
4.0 ml
3.0 G
2.0 ml
0.06 G
50.0 ml

##### Liquid Paraffin

##### Spermaceti

##### Glycerin

##### Propyl hydroxybenzoate

##### Purified Water

Label The Emollient Cream.

Melt the self-emulsifying monostearin and spermaceti with the liquid paraffin and add to the purified water containing the other ingredients which has been heated to about the same temperature. Stir the mixture until cold.

See also Lotion of Hydrocortisone p. 130

#### EXERCISE 10.12

##### Sorbitan monooleate

6 G
3 G
36 G
15 G
40 G

##### Beeswax

##### Soft Paraffin

##### Liquid Paraffin

##### Purified Water

Label The Oily Cream Base.

Melt the first four ingredients together. Add the water, heated to about 60°C and stir the mixture until set.

for the preparation of creams by combining with the following—

- (a) Fatty acids
- (b) Fatty alcohols
- (c) Fatty acid esters of sorbitan

These will now be considered in that order.

**(a) Polyethylene Glycol Esters of Fatty Acids**

These have the general formula



where  $R$  is the fatty acid chain.

Cetostearyl alcohol is commonly used with these compounds as a stabiliser and a suitable emulsifying wax may be prepared as follows—

Polyethylene glycol 600 monostearate	20 parts
Cetostearyl alcohol	80 parts

A proprietary example of this group is Estax 31 which is a polyoxyethylene stearate marketed by the Watford Chemical Co. Ltd (Exercise 10 13).

These esters are incompatible with phenol and phenolic compounds. On long storage they tend to become hydrolysed and emulsions prepared with them are liable to crack although this is unlikely to happen with emulsions which are nearly neutral.

**(b) Polyethylene Glycol Ethers of Fatty Alcohols**

These are compounds of fatty alcohols usually cetyl or stearyl and polyethylene glycol having the following general formula—



The oxyethylene groups ( $n$ ) must number at least 10 if an oil in water emulsion is to be produced and if cetostearyl alcohol is used, the number of  $\text{CH}_2$  groups ( $m$ ) in the alcohol chain will number 15 or 17.

The emulsifying properties of these ethers have been investigated by Hadgraft (1954) who shows that with less than 10 oxyethylene groups the ethers are oil soluble but dispersible in water. These promote water in oil emulsions. The higher water soluble members are dispersible in oil and promote

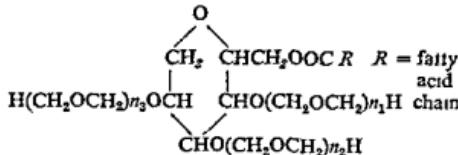
oil in water emulsions. In formulating stable oil in water emulsion creams it is necessary to add a stabiliser such as cetostearyl alcohol.

Cetomacrogol 1,000 B.P.C. is an example of these compounds. The number of oxyethylene groups ( $n$ ) is 20 to 24, the number of  $\text{CH}_2$  groups is 15 or 17 since cetostearyl alcohol is used in the preparation. It is a waxy solid of a cream colour, melting at not less than 38°C. It has a soapy taste and slight fatty and acidic odour. It is soluble in water, alcohol and acetone but insoluble in Liquid Paraffin. It or a similar compound is used in the preparation of Cetomacrogol Emulsifying Wax to the extent of 20 per cent with 80 per cent of cetostearyl alcohol. Cetomacrogol Emulsifying Ointment B.P.C. contains 30 per cent of the emulsifying wax together with soft and liquid paraffins. This ointment is capable of admixture with considerable quantities of water or aqueous solutions to form stable oil in water products.

The polyethylene glycol ethers of the fatty alcohols have similar incompatibilities to the esters previously mentioned but have become much more popular because of greater stability. Unlike the esters they do not become hydrolysed on storage and their content is more reliably uniform.

It should be noted however, that Cetomacrogol is incompatible with phenol and phenolic compounds with tannic acid and it has been claimed by Johnson and Thomas (1955) that quaternary ammonium compounds may be partly inactivated in its presence if the ratio of molecules of cetomacrogol to the quaternary compounds is greater than 4 to 1 (Exercises 10 14 and 10 15).

**(c) Polyethylene Glycol Derivatives of the Sorbitan Esters of Fatty Acids**



**EXERCISE 10 13**

**Estax 31**

10 G

**Soft Paraffin**

40 G

**Stearic Acid**

10 G

**Purified Water**

to 100 G

**Label** The Water miscible Base

Melt the first three ingredients together and add to the Purified Water heated to about 60°C. Stir the mixture until cold.

These have the general formula shown above and it will be seen that they are derived from the sorbitan esters by combination with polyethylene glycols at the non-esterified hydroxyl groups.

Polyisobate 80 of the United States Pharmacopoeia is a polyoxyethylene sorbitan monooleate. It is in fact, a mixture of polyoxyethylene ethers of mixed oleic esters of sorbitan. It is a lemon to amber-coloured oily liquid. It is soluble in water, a 5 per cent solution having a pH of 5 to 7. It is also soluble in fixed oils but not in liquid paraffin. In the formulation of creams it is used with a stabiliser such as cetostearyl alcohol or one of the sorbitan esters.

A series of these compounds is marketed under

the trade name Tween with a number to identify the particular compound. Tween 60, for example, is a polyoxyethylene sorbitan monostearate. As mentioned previously the Tweens are formulated with the Spans to give suitable emulsifying properties in the preparation of creams. The aim in such formulation is to achieve a suitable hydrophilic/lipophile balance (HLB). See p. 70.

Exercise 10.16 is an o/w cream base using these compounds.

Polawax, marketed by Croda Ltd., is a proprietary emulsifying wax consisting of a polyoxyethylene derivative of a sorbitan ester together with cetostearyl alcohol.

#### EXERCISE 10.14

Prepare 60 grammes of Cream of Hydrocortisone B.P.C.

Label as such

Follow the directions given in the Codex.

Cetomacrogol Emulsifying Ointment is also used in Cream of Dimethicone B.P.C. and Cream of Neomycin B.P.C.

#### EXERCISE 10.15

Euflavine	0.05 G
Cetomacrogol Emulsifying Wax	5 G
Soft Paraffin	10 G
Liquid Paraffin	5 G
Purified Water	30 G

Label The Antiseptic Cream

Melt together the Cetomacrogol Emulsifying Wax, Soft and Liquid Paraffins at a temperature of about 60°C. Dissolve the Euflavine in the Purified Water and warm to about 60°C, add the melted mixture and stir until cold.

#### EXERCISE 10.16

Cetostearyl Alcohol	15 G
White Beeswax	2.5 G
*Span 60	7.5 G
*Tween 60	5.0 G
Methyl hydroxybenzoate	0.02 G
Propyl hydroxybenzoate	0.02 G
Purified Water	70 G

Label The Water Miscible Base

Heat the first three ingredients to about 60°C, dissolve the following three ingredients in the Purified Water and heat to about 60°C, add the melted mixture to the solution and stir until cold.

\* Span 60 is a sorbitan monostearate. Tween 60 is a polyoxyethylene sorbitan monostearate (Atlas Powder Co. U.S.A.)

**Anionic****SOAP OR FATTY ALCOHOL SULPHATES AND PHOSPHATES COMBINED WITH FATTY ALCOHOLS**

Anionic emulsifying agents are so called because, on dissociation, the characteristic emulsifying part of the molecule is in the anion. In the case of sodium lauryl sulphate, shown below, the long lauryl chain is in the anion.



Many of the soaps and alcohol sulphates form strong complexes with fatty alcohols to produce excellent oil in water emulsifying waxes.

These have been used in cosmetic and pharmaceutical practice for a number of years and, indeed, include the first of the synthetic waxes which introduced oil in water creams of neutral reaction to dermatological practice. One of these, Lanette Wax, SX was originally a German patent. It consists of cetyl and stearyl alcohols, and 10 per cent of the sulphates of these alcohols. Emulsifying Wax B P is a mixture of cetostearyl alcohol with 10 per cent of sodium lauryl sulphate.

Emulsifying Ointment B P contains 30 per cent of

Emulsifying Wax with Soft and Liquid Paraffins. This anhydrous ointment is capable of admixture with considerable quantities of water or aqueous solutions to form stable oil in water creams. Aqueous Cream B P is an example of this containing about 70 per cent water.

Halden's Emulsifying Base (H E B) is similar to the anhydrous ointments prepared with Lanette Wax SX. It contains cetyl and stearyl alcohols partly phosphated and has the following composition:

Liquid Paraffin	3 parts
Soft White Paraffin	2 parts
Phosphated Alcohols	2 parts

H E B is marketed by Halden (Pharmaceuticals), Ltd.

The anionic emulsifying waxes are incompatible with cationic emulsifying agents such as cetrimide and with cationic compounds such as crystal violet, brilliant green, proflavine, acriflavine, 5 amino acridine and antazoline hydrochloride. They are also incompatible with lead subacetate, iodine, mercuric oxide (if over 2 per cent), and saicylic acid in high concentration.

Exercises 10.17, 10.18 and 10.19 illustrate the use of anionic emulsifying wax creams.

**EXERCISE 10.17**

Prepare 2 ounces of Buffered Cream B N F

Label The Buffered Cream

**EXERCISE 10.18**

Lanette Wax SX	3.0 G
Liquid Paraffin	4.5 G
White Soft Paraffin	12.0 G
Wool Fat	0.2 G
Methylhydroxybenzoate	0.1 G
Glycerin	5.0 ml
Water	20.0 ml

Label The Cold Cream

Melt the Lanette Wax SX, Wool Fat, Soft Paraffin and Liquid Paraffin in a porcelain dish on a water bath, dissolve the methylhydroxy benzoate in the water and, after adding the glycerin, heat to the same temperature as the oily solution. Mix the aqueous and oily solutions and stir until cool.

**EXERCISE 10.19**

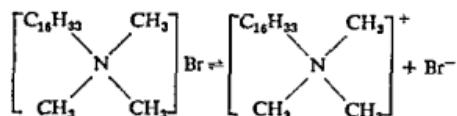
Prepare 50 grammes of Aqueous Cream

Melt the requisite quantity of Emulsifying Ointment prepared in Exercise 10.3. To this add the warm solution of Chlorocresol and stir until cold.

Chlorocresol dissolves much more readily in hot water than in cold. A solution is most readily obtained by shaking it in hot water in a stoppered bottle.

**Cationic****QUATERNARY AMMONIUM COMPOUNDS  
COMBINED WITH FATTY ALCOHOLS**

Cationic emulsifying agents are so called because, on dissociation, the characteristic emulsifying part of the molecule is in the cation. In the case of cetyl trimethylammonium bromide, shown below, the long cetyl chain is in the cation.



The quaternary ammonium compounds are emulsifying agents of the oil in water type. They are not particularly good emulsifying agents when used alone, and for the preparation of emulsion creams they are combined with a stabiliser such as cetostearyl alcohol. The complex formed between the two compounds promotes oil in water emulsions of great stability. It must be stated, however, that the quaternary ammonium compounds are more important for their antiseptic than for their emulsifying properties.

The British Pharmaceutical Codex includes a cationic emulsifying wax consisting of 10 per cent Cetrimide and 90 per cent Cetostearyl Alcohol.

Cetrimide Emulsifying Ointment B P C contains 30 per cent of this wax with 50 per cent White Soft Paraffin and 20 per cent liquid Paraffin. This anhydrous ointment is capable of admixture with at

least an equal quantity of water or aqueous solution to form stable oil in water creams.

The cationic emulsifying agents are incompatible with the anionic types. Also, cetomacrogols may reduce the bactericidal value of quaternary ammonium compounds (See p 142) (Exercises 10.20 and 10.21).

**Barrier Creams**

Barrier creams are preparations which are intended to protect the skin against damage or penetration by various agencies. These may be irritant materials met with in industry, microorganisms, mechanical effects, the ultra violet rays of the sun etc. The great variety of such hazards makes the formulation of suitable creams extremely difficult. A sub-committee of the British Pharmaceutical Codex Revision Committee was appointed to investigate the problem and issued a report containing four provisional formulae which were later modified in the light of suggestions from various sources. These modified formulae are given below. It should be mentioned, however, that these are suggested as basic formulae to be revised, if necessary, in the light of further experience.

**Dust Barrier**

Casein (Rennet, finely powdered)	3
Sodium alginate	2
Glycerin	6
Stearic acid	10
Triethanolamine	155
Chlorocresol	0.2
Phenol	0.5
Distilled water to	100

**EXERCISE 10.20**

Cetrimide	0.5 G
Cetostearyl Alcohol	5.0 G
Soft Paraffin	5.0 G
Liquid Paraffin	15.0 G
Purified Water	25.0 G

**Label: The Antiseptic Cream**

Melt together the cetostearyl alcohol, soft and liquid paraffins. Dissolve the cetrimide in the water warmed to the same temperature (about 60°C). Add the melted mixture to the aqueous solution and stir until cold.

**EXERCISE 10.21**

Prepare one Troy ounce of Cetrimide Cream B N F

Label as such

Follow the Formulary directions

*Water-Miscible Barrier*

Kaolin (sterilised)	20
Fuller's Earth (200 mesh)	3
Hard Soap (powdered)	12
Glycerin	6
Stearic acid	2
Sodium Chloride	1
Chlorocresol	0.2
Phenol	0.5
Distilled water to	100

*Water-repellant Barrier*

Hard Paraffin	25
Soft Paraffin	11.75
Liquid Paraffin	3.5
Cetostearyl alcohol	5
Triethanolamine	0.7
Stearic acid	1.8
Chlorocresol	0.2
Distilled water to	100

*Skin Conditioning Cream*

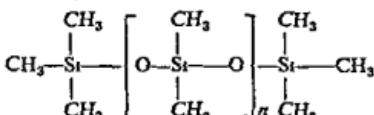
Monostearin, self emulsifying	10
Glycerin	6
Triethanolamine Ricinoleate	1
Wool alcohols	6
Chlorocresol	0.2
Oleyl alcohol	3
Distilled water to	100

*Silicone Creams*

The recent interest in silicones as water-repellent substances for barrier creams has produced a number of proprietary examples and a formula has been included in the British Pharmaceutical Codex

The silicones are organic compounds in which silicone atoms take the place of carbon. In the poly-

methyl siloxanes, for example, there is a chain of alternate silicone and oxygen atoms with methyl groups taking up the additional silicone valencies



Long chain compounds of this type may be cross-linked through oxygen to other chains to form more complex polymers. A wide variety of compounds is available therefore, depending on the extent of polymerisation, giving liquids of varying viscosities.

They are miscible with wool fat, glycerol monostearate, cetostearyl alcohol and polyethylene glycol monostearate, and immiscible with vegetable oils, animal fats, cholesterol, hard paraffin and glycerin, Plein and Plein (1953).

Dimethicone B P C (Synonyms Dimethyl Silicone Fluid, Dimethylsiloxane) is a general title covering a range of polymers which vary in viscosity with increase in molecular weight from 0.65 to 3,000,000 centistokes. Five grades are described in the B P C, viz., 20, 200, 350, 500 and 1,000, the numbers approximating to the viscosity of the product Dimethicone 20, used in the preparation of Dimethicone Cream, is a clear, colourless, odourless liquid. 10 to 30 per cent of this fluid in a suitable base gives protection as a water repellent. Such creams have been used to give protection from napkin rash, bedsores, and to protect the skin around colostomies and ileostomies.

Malloch (1954) describes the use of silicones in the formulation of a number of cream and ointment bases.

**EXERCISE 10.22**

Prepare 60 grammes of Dimethicone Cream B P C

Label Silicone Cream Use as directed

Melt the cetostearyl alcohol with the liquid paraffin and the silicone fluid. Dissolve the cetramide and chlorocresol in the water heated to about 60°C. Add the melted mixture to the solution and stir until cold.

Malloch (1954) describes the use of silicones in the formulation of a number of cream and ointment bases.

**Class 3. Ointments Prepared by Trituration**

This method is used when the base is soft, and the medicament is either a solid insoluble in the base, or a liquid present in small quantity.

**METHOD****STAGE 1**

*Finely powder any solid medicament if necessary*

(see below), sift through a No. 60 sieve, weigh out the required amount, and place on a slab or in a mortar

**STAGE 2**

Weigh out the base, and add a portion of it to the medicament. The portion of base added should be

about three times the weight of the medicament. Triturate thoroughly until a homogeneous product is formed. If a spatula is used, it should be strongly flexed to give considerable pressure.

By this method a smooth ointment is obtained with a minimum of time and labour. If the whole of the base is added, the particles of medicament are scattered throughout a much larger quantity of material, and longer trituration is needed to effect adequate subdivision and even distribution.

A 'concentrated' ointment is easily diluted, and obviously, if it is smooth, the dilution will also be smooth.

#### STAGE 3

Add the remainder of the base, mix well to form a homogeneous product, and then incorporate any liquid ingredients by trituration.

#### Particular Substances

##### LIST B

*Substances commonly prescribed in ointments which are insoluble in the base*

Ammoniated Mercury	Mercurous Chloride
Benzocaine	Opium, powdered
Benzoic Acid	Picric Acid
Bismuth Oxycarbonate	Pyrogallic Acid
Borax	Red Mercuric Iodide
Boric Acid	Red Mercuric Oxide
Calamine	Resorcinol
Chalk, Prepared	Salicylic Acid
Dithranol	Starch
Galls, powdered	Sulphur
Iodoform	Yellow Mercuric Oxide
Lead Acetate	Zinc Oxide
Lead Iodide	

---

#### EXERCISE 10.23

##### Mutter—

Unguenti Zinci Oxiidi B P

3 : 1

Signa More dicto utendum.

Prepare half the pharmacopoeial quantity in grains. Follow the 'Method for Ointments prepared by Trituration' described above.

---

#### EXERCISE 10.24

##### Habeat Unguenti Hydargyri Ammoniati B P

3 : 1

Signa Nomen Proprium

Prepare 500 grains using the previously-made Simple Ointment. Follow the 'Method for Ointments prepared by Trituration' described above. Ammoniated Mercury is a potent substance and inclined to be gritty. Extreme care should be taken in preparing ointments containing it.

---

#### EXERCISE 10.25

##### Recipe—

Acidi Salicylici

ana gr x

Sulphuris Praecipitati

3 : 1

Liquoris Picis Carbonis

ad 3 :

Paraffinum Molle

Fiat unguentum Signa Super ligamentum sindonis extendendum et cruris affecto adhibendum

Adopt one of the following methods to obtain the correct quantity of soft paraffin—

- (a) Measure 1 fluid drachm of Coal Tar Solution in a tared measure, and weigh. The weight of the solution, plus 20 grains, subtracted from 480 grains gives the weight of soft paraffin required.
- (b) Incorporate the ingredients with a quantity of soft paraffin insufficient to produce 480 grains. Transfer to a scale, adjust to 480 grains with soft paraffin, and mix.

The Pharmacopœia sanctions the use of yellow soft paraffin in official ointments containing coloured medicaments, and this rule may be followed for unofficial ointments, therefore use yellow soft paraffin. Follow the 'Method for Ointments prepared by Trituration,' described previously.

---

**EXERCISE 10 26**

Prepare 1 oz of Ointment of Gall and Opium B P C, for rectal use

As mentioned on p 133, 25 per cent of arachis, or olive oil must replace an equivalent amount of a solid base in ointments for rectal use. The base will, therefore, consist of 555 G of lard, plus 185 G of arachis or olive oil, in place of the 740 G of lard prescribed.

Prepare 30 G (the request is for 1 oz *avoirdupois*), and follow the 'Method for Ointments prepared by Trituration'

Dispense in a collapsible tube (p 134)

**EXERCISE 10 27**

*Recipe—*

Picis Carbonis	3 G
Zinc Oxidi	3 G
Paraffinum Molle Flavum	ad 30 G

Fiat unguentum Signa Nocte manequ applicandum

This ointment is a good example of an exercise in mixing. Any carelessness in technique is obvious in the finished preparation because of the colour of the tar and zinc oxide.

Levigate the zinc oxide with a small quantity of the base until smooth. Add the tar and triturate until the concentrated ointment is quite uniform in colour. Gradually add the remainder of the base with thorough trituration.

**EXERCISE 10 28**

Prepare 40 grammes of Calamine Ointment B P C

Label as such

Follow the directions given in the British Pharmaceutical Codex

#### Class 4 Miscellaneous Ointments

##### Iodine in Ointments

This may occur in two forms free, and combined

##### OINTMENTS CONTAINING FREE IODINE

Iodine is only slightly soluble in most fats and oils, but it dissolves in a solution of potassium iodide, forming certain molecular compounds, e.g.  $KI I_2$ ,  $KI 2I_2$ ,  $KI 3I_2$ , according to the concentration, and

these are very soluble in water, alcohol and glycerin. In using a liquid to ensure proper distribution of a medicament it is important that the liquid should be non volatile, otherwise the distributed medicament may crystallise when the solvent evaporates, and these particles in the finished ointment would cause irritation. For this reason glycerin is chosen as the solvent. The use of free iodine in ointments is illustrated in Exercise 10 29.

**EXERCISE 10 29**

Iodine	4 G
Potassium Iodide	4 G
Glycerin	12 G
Wool Fat	4 G
Yellow Beeswax	4 G
Yellow Soft Paraffin	72 G

Prepare and send 50 grammes

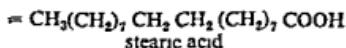
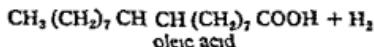
Label Apply to the affected parts as directed

Dissolve the iodine with the potassium iodide in the glycerin using a glass mortar. Melt the other ingredients together over a water bath and stir until set. Add to the solution and mix thoroughly by trituration.

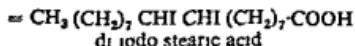
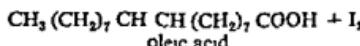
**OINTMENTS CONTAINING COMBINED IODINE**

All fixed oils, and many vegetable and animal fats, are able to absorb iodine under certain conditions. This property is due to the presence of unsaturated bodies which combine with the iodine.

For example, oleic acid contains an olefinic linking, and is, therefore, unsaturated. Upon reduction it yields stearic acid—



Under suitable treatment, one molecule of oleic acid will absorb one molecule of iodine, forming di iodo stearic acid—



Olein, the glyceryl ester of oleic acid, behaves similarly to the free acid in the above respect.

Generally speaking hard, soft, and liquid paraffins are unable to absorb more than a very small proportion (about 2%) of iodine under the conditions of preparing non-staining iodine ointments, because they consist almost entirely of saturated bodies. Most fixed oils, under suitable conditions, can combine with about an equal weight of iodine, a few can absorb much more than this.

The conditions under which iodine ointments of this class are prepared are not those under which the maximum absorption of iodine can be attained, so that the quantity of oil used is much in excess of that required by theory. These ointments are dark, greenish-black preparations, when rubbed into the skin they are readily absorbed and leave no stain, hence they are called non staining iodine ointments.

**METHOD OF PREPARING  
NON-STAINING IODINE  
OINTMENTS**

Finely powder the iodine and place in a closed vessel, e.g. a stoppered wide mouthed bottle, with the other ingredients.

Heat on a water-bath at a temperature not exceeding 60°C, stirring occasionally, until combination has taken place. The product should be greenish-black upon completion, and not brown, the time required for this varies, but may be 2 to 6 hours.

Transfer to a pot and allow to cool without stirring, as this renders the product opaque due to the incorporation of air (Exercise 10.30).

**Ointments Containing Metallic Oleates**

Metallic oleates were once considered desirable by dermatologists, since they are soluble in many ointment bases and, therefore, offer better dispersion than oxides and insoluble salts. There are no examples in the B.P. or B.P.C. Oleated Mercury of the B.P.C. 1958 has been deleted from the present B.P.C. (Exercise 10.31).

**EXERCISE 10.30***Recipe—*

Iodi	gr xx
Acidi Oleici	gr xl
Olei Ricini	gr ix
Paraffinum Molle Flavum	ad ʒ j

Misce ut fiat unguentum denigrescens

Signetur Applicetur omni nocte

Follow the above method. Note that an assay is required for Non-staining Ointment of Iodine B.P.C.

**EXERCISE 10.31**

Prepare 20 grammes of Oleated Mercury B.P.C. 1958

Follow the B.P.C. 1958 directions. It will be found that upwards of an hour is required to effect combination of the oxide with the oleic acid.

Combination may be greatly accelerated by heating on a boiling water bath, stirring continuously. The higher temperature is not detrimental provided there is continuous trituration. When all the oxide has combined,

usually after a few minutes, the dish must be immediately transferred to a bath of cold water, and rapidly cooled. Repeat the preparation as described, using the higher temperature, and compare the products.

A common fault in preparing Oleated Mercury is to fail to get all the yellow oxide into combination with the acid. Small quantities, such as the above, should be made in a large evaporating basin using a pestle and a vulcanite spatula to manipulate the

ingredients. The contents of the basin should not be allowed to come within about an inch of the rim of the dish and the spatula should not be scraped on its rim. Thus, all the oxide is kept in contact with the acid until the reaction is complete.

## PASTES AND JELLIES

These usually differ from ointments in having a non-greasy base. The character of the base forms a useful basis for classification, thus—

Class 1 Pastes with a Gelatin Base  
Class 2 Pastes with a Starch Base

Class 3 Pastes with a Tragacanth Base  
Class 4 Pastes with Polyethylene glycol Base  
Class 5 Pastes with a Cellulose Derivative Base  
Class 6 Pastes with a Pectin Base  
Class 7 Pastes with a Solid Colloid Base

### Class 1 Pastes with a Gelatin Base

A hot solution containing only 20 per cent of gelatin sets to a jelly upon cooling and this property is used in preparing pastes to be applied to the skin. They usually contain sufficient gelatin to form stiff pastes which are applied in a melted condition with a stiff brush. To prevent putrefaction it is necessary to add a preservative, and 20–40 per cent of glycerin fulfils this object, as well as supplying a non volatile, hygroscopic emollient.

Gelatin pastes are medicated with various substances, e.g. Zinc Oxide (10–15 per cent), Ichthammol (5 per cent or more), Iodoform (10 per cent), Salicylic Acid (10 per cent), and combinations of these.

### METHOD

Sheet or powdered gelatin may be used. Sheet gelatin should be cut into small pieces with scissors

before placing in water to speed up solution. Powdered gelatin should be carefully sprinkled on the surface of the water and stirred in to avoid the formation of lumps which may be difficult to disperse. The gelatin and water, in a tared dish, is set aside for about 15 minutes. This allows the gelatin to soften and swell, thereby hastening subsequent solution. Add the glycerin, and record the weight of the contents of the dish.

Heat over a water bath and stir occasionally until the gelatin dissolves. Then adjust the product to the original weight by the addition of water.

Incorporate the medicament, and stir until the product is viscous. If the medicament is a solid it must be finely powdered and sifted through a No. 60 sieve before use.

### EXERCISE 10 32

#### Prepare 50 grammes of Unna's Paste \*

Zinc Oxide	15
Gelatin	15
Glycerin, by weight	35
Water, by weight	35

Follow the general directions given above, thus—

Soak the gelatin in the water in a tared dish for about 15 minutes. Then add the glycerin and record the weight. Heat over a water bath until the gelatin dissolves, transfer the dish to a scale and adjust to the original weight with water. Finally, add the zinc oxide, previously sifted, stir well, pour into a pot, and stir occasionally until viscous.

\* Now official under the name Zinc Gelatin

(continued overleaf)

**Exercise 10.32 continued**

In use, the jelly is melted and a suitable quantity is applied, but sedimentation of insoluble medicament can occur. A more useful and elegant method of packing is therefore, to pour the molten mass into a tray just before setting and, when set, to cut with a sharp knife into small pieces about  $\frac{1}{2}$  in square. Small pieces are to be preferred to large as, in use, a suitable number can be melted for one application. The pieces may be placed in a wide mouthed jar or an ointment pot.

**Class 2 Pastes with a Starch Base**

There are two kinds of starch pastes—

**(a) Pastes Containing Gelatinised Starch**

These contain only about 10 per cent of starch—a proportion which will not yield a paste unless the starch is gelatinised. A simple starch jelly would rapidly ferment, hence, a large proportion of glycerin is usually included to serve the three fold purpose of preservative, anti-drying agent, and emollient—as in gelatin pastes. Water must be present, otherwise the starch would not be gelatinised.

Starch pastes are frequently medicated, as mentioned above for gelatin pastes.

**METHOD**

Heat the glycerin to 140°C in a dish over a Bunsen flame, and mix the starch with the water in a small mortar. Add the starch and water mixture quickly to the hot glycerin, stir vigorously and continue to heat for about 1 minute, and then remove the burner.

The product should be a soft, spreadable translucent homogeneous jelly. When cool incorporate the medicament, if required, by trituration.

An alternative method is to mix the starch, glycerin and water together, and heat rapidly over a Bunsen flame until the starch gelatinises.

Some water is lost in both methods but the loss in the latter method is more variable than in the former, with resultant variation in the consistency of the jelly—the first method should therefore be adopted (Exercise 10.33).

**(b) Pastes containing Ungelatinised Starch**

In these the starch functions as a mechanical stiffening agent, and a large proportion must be present.

**METHOD**

Dissolve any water-soluble substances present in the water, mix the starch with any insoluble ingredients and then add the aqueous liquid mix thoroughly to form a homogeneous paste.

**EXERCISE 10.33****Recipe—**

Resorcinolis	3%
Ichthammolis	5%
Glycerinum Amyli	ad $\frac{1}{2}$ t

Fiat pasta Signetur Parti affectae applicanda

The B.P.C. quantities for Starch Glycerin give a total of 1,000 grammes, but some loss of water occurs during preparation, so that the product weighs considerably less than this—therefore prepare one-twenty fifth of the quantity, and follow the first of the methods described above.

Using Starch Glycerin, prepare 500 grains of the prescribed paste by trituration.

**EXERCISE 10.34****Recipe—**

Ichthammolis	5 G
Resorcinolis	1 G
Phenolis	1 G
Amyli pulverati	15 G
Aquam	ad 30 G

Follow the above directions, thus—

Dissolve the Ichthammol, Resorcin, and Phenol in 8 ml of water and incorporate the solution with the Starch.

## Class 3. Pastes with a Tragacanth Base

A component of tragacanth, called bassorin, swells with water to form a jelly, hence the name bassorin paste sometimes applied to tragacanth jellies

A lumpy product is formed if water is added directly to powdered tragacanth—the surface particles swell and prevent access of water to those in the centre. A distributive agent is, therefore, necessary to ensure free swelling throughout the powder, and alcohol or glycerin answers this purpose, and also acts as a non-drying preservative. Water and glycerin are both essential in a tragacanth paste, for which a suitable formula is given below—

Bassorin	Tragacanth, in powder	5
Paste	Alcohol	10
	Glycerin, by weight	2
	Water, by weight	to 100

## METHOD

Triturate the tragacanth with the alcohol in a mortar and add, all at once, about 70 parts of the water and triturate briskly. Add the glycerin and the remainder of the water with stirring.

Similar preparations without alcohol may be made by triturating the tragacanth with the glycerin which also acts as a distributing agent.

Some formulas contain gelatin as well as tragacanth, glycerin and water. They are prepared by combining the above method with that for gelatin pastes, i.e. the gelatin is softened in the water, the tragacanth mixed with the glycerin, added to the softened gelatin, the mixture heated and stirred until the gelatin dissolves, and then finally adjusted to the correct weight. Heating assists the tragacanth to form a more viscous paste.

## EXERCISE 10.35

Dithranol	0.1%
Bassorin Paste, a sufficient quantity	
Send 2 oz Label To be used as directed	
Prepare 1,000 grains of bassorin paste as above, and from this prepare 1,000 grains of the medicated paste, to the following formula—	
Dithranol    1 grain Bassorin Paste                                    999 grains	
Incorporate the Dithranol by trituration	

## Class 4. Pastes with a Cellulose Derivative Base

Cellulose ethers, produced artificially, have come into wide use in recent years for the preparation of jellies. These cellulose jellies are clear and transparent, neutral, they keep indefinitely, and are simple to make. On the other hand, they are moderately expensive compared with tragacanth or starch jellies, and are incompatible with certain medicaments, for instance, a satisfactory tannic acid jelly cannot be prepared with a cellulose ether jelly.

As mentioned on p. 62, they are usually methyl or ethyl ethers or mixed methyl-ethyl ethers. They are soluble in cold water, a mucilage or jelly being conveniently made by stirring the ether into the required

volume of water and allowing to stand overnight. Alternatively, a mechanical stirrer may be used to hasten solution. Other cellulose derivatives are salts of cellulose carboxy acids, and are more readily soluble in hot water.

The various proprietary brands on the market vary in the viscosity of their solutions, and it is not possible to give a general direction for the preparation of jellies. Also, since most manufacturers supply several grades, care should be taken to obtain all the necessary information before using a particular product. Cellofas B, a carboxymethyl cellulose, (made by I.C.I.) is used in Exercise 10.36.

## EXERCISE 10.36

Cellofas B	4.00 G
Eusflavine (or Proflavine)	0.05 G
Distilled Water	46.00 G
Label Eusflavine (or Proflavine) Jelly, 1 in 1,000	

(continued overleaf)

**Exercise 10.36 continued****METHOD**

Dissolve the Euflavine (or Proflavine) in the water without heating, place the solution in a mortar (preferably glass, to facilitate observation), add the Cellosol B, stir thoroughly for 10-15 minutes, set aside and stir occasionally until a clear jelly is formed

**Class 5. Pastes with a Pectin Base**

Pectin is a carbohydrate of complex nature and is obtained from fruits, e.g. apples. It is used commercially for the preparation of jams and jellies. It dissolves in water to form a gel and commercial varieties of different 'setting' value are made. Pure forms are of use for medicinal purposes, e.g. the treatment of diarrhoea. It is also used in combina-

tion with acacia and tragacanth as an emulsifying agent.

The quality used for jams and jellies is usually described as '100-Grade'. Medicinal quality is described as '200-Grade'.

The following exercise illustrates its use for the preparation of a pharmaceutical jelly.

**EXERCISE 10.37**

Sulphanilamide	0.6 G
Pectin	4.5 G
Glycerin	12.0 G
Normal Saline Solution	42.9 G

**Label** The Sulphanilamide Jelly

Triturate the sulphanilamide and the pectin with the glycerin until smooth. Add all at once, with continuous stirring to prevent the formation of lumps, the Normal Saline Solution heated to boiling.

**Class 6 Pastes with a Solid Colloid Base****Bentonite**

This is a mineral clay, or 'soap clay,' of volcanic origin which receives its name from the deposits near Fort Benton in the Missouri Valley. It consists of the hydrous silicates of magnesium, calcium and aluminium with traces of iron, with the following analysis— $(\text{Mg Ca})\text{O Al}_2\text{O}_5, 5\text{SiO}_2, n\text{H}_2\text{O}$ .

Purified forms are used for pharmaceutical preparations, both external and internal, as suspending and emulsifying agents.

With water it swells to a gelatinous viscous mass, a 25 per cent suspension having the consistency of anhydrous wool fat.

An ointment base may be made consisting of

**Aluminum Hydroxide**

Colloidal hydrated aluminum hydroxide, marketed under the name of Unemul by Universal Emulsifiers, Ltd., Dorset, has a number of industrial applications as an emulsifying, wetting and suspending agent. It forms oil-in-water emulsions.

It is non toxic, is not affected by bacteria, and requires no preservative.

Of special value is its property of assisting the wetting of substances such as sulphur which are not readily miscible with water.

Exercise 10.38 is a semi liquid preparation described by Swallow and Whittet in the *PJ*, Dec 20, 1941.

**EXERCISE 10.38**

Sulphanilamide	5 G
Unemul	5 G
Glycerin	45 ml
Cod Liver Oil	45 ml

**Label** The Sulphanilamide Cream.

Mix the sulphanilamide and Unemul with the glycerin. Stir in the cod liver oil and pass through a hand homogenising machine.

Bentonite 10 per cent to 15 per cent in water, but it tends to dry on the skin Glycerin is sometimes added therefore as an humectant

In preparing gels water should not be added to bentonite alone, as a lumpy product results. The bentonite should be sprinkled on the surface of hot water and left for about twenty four hours. A satisfactory aqueous dispersion may, however, be made if the bentonite is first triturated with glycerin

or intimately mixed with other powders such as zinc oxide or calamine

Exercise 10.39 is an example of a water miscible ointment base in which the Bentonite acts as a stabiliser. If Bentonite is to be used in a cream for application to open wounds it should first be sterilised in a hot air oven for an hour at 150°C.

Exercise 10.40 is an example of its use as a base for a paste

#### EXERCISE 10.39

Bentonite	3 parts
Lanette Wax SX	4 parts
Soft White Paraffin	10 parts
Liquid Paraffin	20 parts
Distilled water	to 100 parts

Melt the Lanette Wax SX, Soft White Paraffin and Liquid Paraffin over a water bath. Disperse the Bentonite in the product and add the water, previously heated to a similar temperature. Stir until cold

#### EXERCISE 10.40

Zinc Oxide	10 G
Glycerin	10 G
Bentonite	10 G
Distilled Water	to 100 G

Label The Paste

Mix the zinc oxide and the Bentonite and triturate with the glycerin Add the distilled water gradually with continual trituration

#### Selection of Base for Ointments

Much attention has been given of late to a consideration of the vehicles by which medicaments are applied externally. This is particularly so in the case of ointment, paste and jelly bases, and, although the problems are by no means resolved, much interesting work has been done.

Various bases are in use and new ones are being evolved continuously. They include greasy bases, anhydrous emulsifying bases which readily absorb water and aqueous solutions, emulsion bases of both water in oil and oil in-water types, and also aqueous non greasy bases.

There can be no ideal ointment base, since different types are required for different purposes. For example, a base which is suitable for an intact skin may not be satisfactory for a broken skin. Again, a base which meets the requirements of a dry skin may not be suitable for a greasy skin.

These and other related problems are for the dermatologist to resolve, but the pharmacist must have a good understanding of the problems involved if he is to be in a position to advise on the properties of the various bases.

The more important factors are briefly discussed below

#### Dermatological Factors

##### 1 ABSORPTION AND PENETRATION

These terms are commonly used indiscriminately and should be carefully distinguished.

Harry (1948) has suggested that absorption should be reserved for indicating actual entry into the blood stream, i.e. systemic absorption and the word 'penetration' for passage through the skin, i.e. cutaneous penetration.

The student will remember from his studies in physiology that the skin is not simply a covering for the body but a complex living tissue containing several differentiated layers numerous sebaceous glands, sweat pores and hair follicles. Penetration of the skin by an ointment appears to occur by way of the sweat glands, sebaceous glands and down the hair follicles. The non-keratinised cells at the base of the hair follicles is the most likely point of penetration. It has also been suggested that some diffusion through the epidermis also occurs.

It is probable that ointment bases are not, themselves, absorbed into the blood stream although they may penetrate into the tissues more or less deeply. On the other hand, it has been generally considered that bases which penetrate deeply will assist the systemic absorption of the medicament. This may well be true, but it should be remembered that systemic absorption is undoubtedly to a large extent a property of the medicament itself.

The various experiments which have been conducted to elucidate problems of absorption and penetration may be grouped under three headings, as follows—

- (a) Penetration of the *ointment base* through the skin.
  - (b) Absorption of the *medicament* into the blood stream.
  - (c) Rate of release of the medicament from the base
- (a) Wild (1911) carried out simple quantitative experiments with different bases by rubbing weighed quantities into definite areas of skin for a fixed time. The unabsorbed base was carefully collected from the skin and weighed. The difference gave the amount of base absorbed.

Strakosch (1943) made histological examinations of human skin biopsies at intervals for 24 hours after treatment with the base to determine the amount of penetration.

In each case it was found that paraffins did not readily penetrate the skin but that lard did. This was in accordance with the belief that animal fats and oils penetrate but that mineral oils do not. Strakosch also claimed, however, that an emulsion of liquid paraffin had good penetrating properties. He found that an emulsion of liquid paraffin and arachis oil in water, emulsified with triethanolamine and containing stearic acid and cetyl alcohol, gave most complete penetration. Wool fat emulsions were also shown to penetrate readily.

(b) Various workers have attempted to estimate systemic absorption following inunction by estimating the amount of medicament in the urine and blood stream.

Bliss (1936) used various ointment bases and methyl salicylate as medicament. His experiments were conducted with human subjects and he estimated the salicylates in the urine after rubbing in definite weights of the ointments under standard conditions. He found that no difference of therapeutic significance could be demonstrated between a wide range of common bases. He similarly conducted experiments using iodine, potassium iodide

and quinine hydrochloride instead of methyl salicylate with similar results.

Zeigler (1928), using both dogs and human subjects, showed that iodine is absorbed even from a paraffin base.

More recently, Johnson and Lee (1943) using radioactive sodium chloride as the medicament, found that lard and water miscible bases gave more rapid absorption than hydrocarbon bases. The radioactive substance was measured in the tissues and in the urine.

(c) A number of methods, chiefly of the *in vitro* type, have been devised to show the rate of release of a medicament from an ointment base, and although these may not indicate what happens when the ointment is applied to the skin they form useful methods of comparison and throw a good deal of light on the subject. A review of this aspect has been published by Gemmel and Morrison (1957).

In the pigmentation of the skin with sodium fluorescein in various ointment bases, Skinner (1930) found that the extent of coloration varied widely, depending on the base used. The least effect was obtained with a paraffin base and the best with a base containing the following ingredients: Hydrous wool fat, 250, oleic acid, 30, water, 100, glycerin, 20, tragacanth, 2.5. Intermediate pigmentation was obtained with hydrous wool fat and olive oil, lard, cold cream. Skinner suggested that if this were carried out under standard conditions, viz. a stated weight rubbed into a defined area, it might give a clue to the absorption of the base.

Several workers have used the now familiar agar cup and agar plate methods to show the rate of release of bactericidal substances from various bases. In these methods solid agar bacterial culture medium contained in a Petri dish is used, inoculated with a culture of an organism such as *Staphylococcus aureus*. A little of the ointment is placed on the surface or in a hole cut in the medium. On incubation at 37°C the organism will grow except where the bactericide has diffused outward through the medium, forming a zone of inhibition. The diameter of this zone is a measure of the release of the bactericide from the base.

In general, the results of these experiments have shown that water miscible bases give a much greater zone of inhibition than greasy bases or even water-in-oil emulsions.

Bandelin and Kemp (1946) smeared test tubes internally with thin layers of sulphonamide ointments and found the rate of release of the sulphonamide by filling the tubes with saline or serum and, after a period of incubation, estimating the amount of the

sulphonamide in the solutions. They also found that oil-in-water emulsions were superior to greasy bases or to water-in-oil emulsions.

Waud and Ramsay (1943) used as a diffusing medium a 4 per cent agar gel containing Ehrlich's solution. A quantity of this was placed in each of a number of test tubes and covered in each case with a layer of ointment containing a sulphonamide. The release and diffusion of the sulphonamide was measured by the red colour which developed in the agar gel. Once again the superiority of the oil-in-water emulsion bases in such circumstances was demonstrated.

Rae (1944) used ointments containing sodium chloride and placed these in glass tubes closed with a cellophane membrane. These were then suspended in water and the sodium chloride which diffused through the membrane was measured. The following figures, representing the number of millilitres of N/10 silver nitrate required to react with the released sodium chloride, are comparative for the different bases used. Simple Ointment B.P., 0.4, Lanette Wax SX, 10 per cent in distilled water, 4.0, 25 per cent soft paraffin with 10 per cent methyl cellulose in distilled water, 12.6, pectin 5 per cent, 23.8. This is yet another result showing the more rapid release of medicament from aqueous or water-miscible bases.

Gemmell and Morrison (1958) found that oil solubility increased the skin penetration of drugs but they claim that the vehicle is less important than the nature of the drug, and this is supported by the evidence of Peck (1950) and his associates. Shelley and Melton (1949) showed that histamine penetrated most fully in aqueous solution. It would seem that substances with both oil and water solubility are most readily absorbed.

Ionisable substances seem to be prevented from penetrating the skin by a barrier in the epidermis, and it has been suggested by Marriott (1958) that this may be overcome by using the corresponding esters. Methyl salicylate, for example, readily penetrates the intact skin.

The above summary of research into these questions is by no means exhaustive and further facts are being elucidated continuously. However, certain conclusions can be reached, and it will be helpful to summarise them.

Paraffins do not readily penetrate the skin.

Animal and vegetable fats and oils do penetrate the skin.

Wool fat, especially when combined with water, penetrates the skin.

Absorption into the blood stream may be very largely a function of the medicament.

Substances with both oil and water solubility are most readily absorbed.

Water soluble substances appear to be most readily absorbed from aqueous bases.

Oil-in-water emulsion bases release their contained medicament more readily than greasy bases or water-in-oil emulsion bases.

## 2 EFFECT ON SKIN FUNCTION

A criticism of the greasy bases is that they interfere with normal skin functions, e.g. heat radiation and sweat excretion. Oil-in-water emulsions and other water-miscible bases are much less open to this criticism, having a cooling rather than a heating effect and mixing readily with the aqueous skin secretions. Greasy bases, moreover, may prove irritant.

## 3 MISCELLIBILITY WITH SKIN SECRETIONS AND SERUM

The skin produces both aqueous and fatty secretions and these are more readily miscible with emulsion bases than with greasy bases. This miscibility results in a more rapid and complete release of medicament to the skin. In consequence, when such bases are used, less medicament is necessary.

Oil-in-water emulsions will mix more readily with serum from broken surfaces, and they have also been claimed to be particularly useful in such pathological conditions as weeping eczema.

## 4 COMPATIBILITY WITH SKIN SECRETIONS

An average figure for the pH of the skin is 5.5 and the reaction of an ointment base should not differ from this figure to any appreciable extent. The tendency is to use neutral ointment bases as they do not cause trouble in use and avoid incompatibility with medicaments.

## 5 FREEDOM FROM IRRITANT EFFECT

Ointment bases should not have any irritant effect on the skin and those in general use are non-irritant, although it has been said that on some skins greasy bases cause oedema.

In selecting emulsifying agents for the preparation of emulsion bases this must be constantly borne in mind. Dodd, Hartmann and Ward (1946) examined nine surface-active agents for this purpose and found that four were irritating, all four being ionic substances. The tests were carried out on rabbit eye and skin and also on human skin. Green, on the other hand, found that a water-miscible paraffin base emulsified with cetyl trimethyl ammonium bromide and cetyl alcohol caused less irritation than other

oil-in-water bases when injected into the thigh muscles and under the abdominal skin of rats

It has been suggested that paraffins which penetrate the skin, as they do in emulsified form, may be harmful since they remain unabsorbed

This factor is one which requires further investigation, especially in regard to the newer bases. It is very clear, however, that all bases should be of a high standard of purity and that bases, especially those for eye ointments, should be free from foreign particles

#### 6. EMOLlient PROPERTIES

Dryness and brittleness are common conditions of the skin which, in addition to causing discomfort, impair its protective properties against micro-organisms and harmful chemicals

Water will, of course, soften the skin, and humectants such as glycerin and other hygroscopic substances will help to keep the surface soft and moist. Ointments containing wool fat and lard, which penetrate the skin, have long been used as emollients. As paraffins, which do not readily penetrate the skin, are also emollient the explanation of how they act is not simply that they oil the horny layer. It has been shown by several research workers that these substances keep the skin soft by preventing a too rapid loss of moisture. Blank (1952), Peck and Glick (1956) and Powers and Fox (1958) have all shown that this is how the emollient effect is achieved. Continuous hydration of the horny layer is due to the conveyance of moisture from deeper layers in the skin. Normally this is sufficient to keep the skin in a supple and pliable condition. If, however, the horny layer becomes dehydrated the loss may not be made good and dryness and brittleness occurs.

#### 7 EASE OF APPLICATION AND REMOVAL

This may be a matter of first importance where damage may be caused to new-formed tissue by the application of an ointment having a stiff or sticky consistency. Thin oily or aqueous lotions may be safely applied, but, if an ointment is required, one of the emulsion type is much more easily spread over the area.

In addition, emulsion bases, particularly the water-miscible type, are readily removed by simply washing with water. In this connexion scalp ointments prepared with a water-miscible base are particularly useful because of the ease with which they can be removed from the hair.

Zopf (1945) claims that soft paraffin should be included in all such bases to give smoothness and 'slip'. This, it is claimed, aids spreading and gives

a softening effect to the film of ointment on the skin.

In addition to the above factors, others which should be kept in mind are the degreasing effect of oil absorbing emulsion bases and the dehydrating effect of water-absorbing bases.

#### Pharmaceutical Factors

These factors include the provision of bases to meet the requirements of the dermatological factors already mentioned, also the physical and chemical factors such as stability, solvent and emulsifying properties and consistence. The aim should be to provide ointment bases which meet with all essential medical requirements and are pharmaceutically elegant.

#### 1 STABILITY

Animal and vegetable fats and oils are subject to oxidation unless they are suitably protected. In the past, lard was one of the common ointment bases but, as it became rancid on storage, it was converted to Benzoinated Lard by digestion with Siam Benzoin. This product was reasonably stable due to the presence of cinneryl benzoate an oil soluble constituent of the benzoin. The B.P.C. 1963 directs that lard should be protected with 0·01 per cent of butylated hydroxytoluene as antioxidant, together with 0·01 per cent of citric acid as synergist.

Free acids in vegetable oils may cause trouble with oil in water creams containing zinc oxide since the zinc-fatty acid soap which forms will tend to reverse the emulsion. This difficulty may be avoided by prior trituration of the zinc oxide with water.

Soft Paraffin is inert and perfectly stable with ointment medicaments, and the same applies to Simple Ointment and Paraffin Ointment. Liquid Paraffin is liable to oxidation if stored for long periods with the production of peroxides, and the Pharmacopoeia allows the use of tocopherol or butylated hydroxytoluene as antioxidants up to 10 parts per million.

Wool Fat preparations are usually stable but emulsions made with it are liable to surface discolouration.

The Pharmacopoeia requires Wool Alcohols to be stored in a well-closed container, protected from light, and in a cool place. This is important as it is liable to surface oxidation. It should be kept in pieces as large as possible and not broken until required for use. Serious loss of emulsifying power occurs if the wax is badly stored for any length of time. Clark and Kitchen (1960) have shown that Wool Alcohols may be protected by the antioxidant

butylated hydroxy anisole, 500 parts per million (see Appendix) The Pharmacopoeia requires between 500 and 1000 parts per million of butylated hydroxyanisole or butylated hydroxytoluene It also includes a limit for copper since this catalyses the oxidation

The emulsified bases present special problems Separation of the phases may occur under unfavourable conditions of temperature or by imperfect formulation Oily Cream, a Wool Alcohol, stabilised emulsion is liable to separation although easily reemulsified by stirring

Oil-in-water creams present a good medium for the growth of microorganisms and, therefore, require a preservative (see p 69) Containers are obviously important, and metal closures must be avoided with oil-in-water creams or even water-in-oil creams since separation may occur The incompatibilities of the emulsifying agents present another problem These are mentioned under the individual substances

## 2 SOLVENT PROPERTIES

Most medicaments in ointments are insoluble in the base, being present in finely divided form throughout the base There are some cases in which this condition would be unsatisfactory To take an example, phenol, in solid form is caustic, and its presence in a finely-divided form might cause blisters The base selected for Phenol Ointment B P 1948 must therefore be capable of retaining the phenol in solution The base selected is a mixture of hard and soft paraffins, beeswax and lard Phenol is not very soluble in hydrocarbons, and 3 per cent, the proportion of phenol in the official ointment, would not remain in solution in a purely hydrocarbon base Phenol is much more soluble in animal or vegetable fats and oils, and therefore the inclusion of a suitable proportion of lard ensures solution of the phenol Compound Mercury Ointment B P C 1958 is another example—this ointment contains an appreciable proportion of olive oil to effect solution of the camphor

## 3 EMULSIFYING PROPERTIES

Hydrocarbon bases possess only feeble water-absorptive properties, and are able to 'take up' aqueous or non-fatty liquids, e.g. alcohol, glycerin, to only a small extent It is difficult to incorporate more than a small proportion of aqueous or alcoholic liquids with hydrocarbon bases alone, and such admixtures are almost invariably mechanical emulsions, i.e. the sub-divided globules of aqueous or alcoholic liquid are prevented from coalescing merely by the viscosity of the base

On the other hand, some animal fats absorb water to an appreciable extent, thus wool fat takes up about 50 per cent of water, and when mixed with other fats, e.g. soft paraffin or lard, it is able to take up several times its own weight of aqueous or hydro-alcoholic liquids, and most of these mixtures are true emulsions This property of wool fat furnishes one reason for its inclusion in the base for eye ointments, discussed below As previously explained Ointment of Wool Alcohols is able to take up large amounts of water or hydro-alcoholic liquids

Emulsions of Wool Fat and Wool Alcohols are of the water-in-oil type The three emulsifying ointments—Emulsifying Ointment B P, Cetrimide Emulsifying Ointment B P C and Cetomacrogol Emulsifying Ointment B P C, are all capable of taking up considerable quantities of water or aqueous solutions forming oil-in-water creams

## 4 CONSISTENCE

The official ointment bases are designed to produce ointments of suitable consistence at temperatures prevailing in this country—substances with too low a melting-point being stiffened by adding a proportion of a substance with a higher melting-point Thus, hard paraffin is included in Ointment of Wool Alcohols, and Simple Ointment, and beeswax is used in the preparation of Paraffin Ointment See also p 133 regarding the consistency of rectal ointments and p 160 which describes eye ointment base

## REFERENCES

- BLISS, A R (1936) Absorption from the Human Skin *J Amer Pharm Ass*, 25, 116
- BANDELIN, F J and KEMP, C R (1946) The Evaluation of Various Bases for Sulphonamide Ointments *ibid*, 35, 65-71
- BLANK, I (1952) Factors which Influence the Water Content of the Stratum Corneum *J Invest Dermat*, 18, 433-440
- CLARK, E W and KITCHEN, G F (1960) Stability of Oily Cream *J Pharm Pharmacol*, 12, 227
- DODD, M C, HARTMANN, F W and WARD, W C (1946) Surface-active Agents as Ointment Bases *J Amer Pharm Ass*, 35, 33-41
- GREEN, A F (1946) The Pharmacological Assessment of Antibacterial Creams *Quart J Pharm*, 19, 106-112
- GEMMELL, D H O and MORRISON, J H (1957) Drug Release from Topical Applications *Pharm J*, 179, 47
- GEMMELL, D H O and MORRISON, J H (1958) The Percutaneous Absorption of Sulphanilamide *J Pharm Pharmacol*, 10, 167-174

- GEMMELL, D. H. O and MORRISON, J. H. (1958) Comparative Studies on Percutaneous Absorption. *Ibid.*, 10, 553-560
- GEMMELL, D. H. O and MORRISON, J. H. (1958) Factors Influencing Percutaneous Absorption. *Ibid.*, 10, Supp., 210T-212T
- HADGRAFT, J. W. (1954) The Emulsifying Properties of the Polyethylene Glycol Ethers of Cetostearyl Alcohol. *J Pharm Pharmacol.*, 6, 816
- HARRY, R. G. (1948) The Principles and Practice of Modern Cosmetics Vol. II (Leonard Hill)
- JOHNSON, G. W. and LEE, C. O. (1943) A Radioactive Method of Testing Absorption from Ointment Bases. *J Amer Pharm Ass.*, 32, 278-280
- MALLOCH, M. M. (1954) Silicones in Creams and Ointment Bases. *Pharm J.*, 173, 140
- MARRIOTT, R. H. (1958) Penetration of Skin—Dead or Alive. *J Soc Cosmet Chem.*, 9, 229-242
- PECK, S. M. and GLICK, M. D. (1956) A New Method for Measuring The Hardness of Keratin. *Ibid.*, 7, 530-540
- PECK, S. M., FINKLER, B., MAYER, G. G. and MICHELFELDER, T. (1950) Effects of Various Modes of Administration of Pyribenzamine on the Histamine Wheal and Epidermal Sensitivity Reactions. *J Invest Dermat.*, 14, 177-191
- PLEIN, J. B. and PLEIN, E. M. (1953) Preliminary Study of Silicone Oils as Dermatological Vehicles. *J Amer Pharm Ass (Sci Ed.)*, 42, 79
- POWERS, D. and FOX, C. (1958) Effects of Cosmetic Ingredients and Preparations on Moisture Loss from the Skin. *Drug and Cosmet Ind.*, 82, 32-33
- PRICE, J. C. and OSBORNE, G. E. (1958) An Evaluation of Methods Used in the Preparation of Fused Ointment Bases. *J Amer Pharm Ass (Prac Ed.)*, 19, 679
- RAE, J. (1944) Method of Testing Absorption from Various Ointment Bases. *Brit J Dermat.*, 56, 92-94
- SCHULMAN, J. H. and COCKBAIN, E. G. (1940) Molecular Interaction at the Oil/Water Interfaces Part I Molecular Complex Formation and Stability of Oil in water Emulsions. Part II Phase Inversion and Stability of Water in-oil Emulsions. *Trans Far Soc.*, 36, 651, 661
- SHELLEY, W. B. and MELTON, F. M. (1949) Factors Accelerating the Penetration of Histamine through Normal Intact Human Skin. *J Invest Dermat.*, 13, 61-71
- SKINNER, H. (1930) Ointment Bases. Practical Notes on the Preparation of Medicaments of this Class and their Absorption. *Chemist and Druggist*, 112, 795-796
- SPLATON, L. M. (1956) *Pharmaceutical Emulsions and Emulsifying Agents* (Chemist and Druggist)
- STRALOSCH, E. A. (1943) Studies on Ointments I Penetration of Various Ointment Bases. *J Pharmacol Exp Ther.*, 78, 65-71
- SYMPORUM SESSION PHARMACEUTICAL CONFERENCE (1947) New Emulsions and Ointment Bases. *Quart J Pharm.*, 20, 484
- WILD, R. B. (1911) On the Official Ointments with Special Reference to Substances Used as Bases. *Brit med J.*, 2, 161-162
- WAUD, R. A. and RAMSAY, A. (1943) Diffusion of Sulphonamides out of Certain Bases. *Canadian med. Ass J.*, 48, 121-123
- ZEIGLER, W. H. (1928) A Study of the Absorption and Antiseptic Properties of Several Types of Iodine Ointments. *J Amer pharm Ass.*, 17, 648-650
- ZOFF, L. C. (1945) Hydrophilic Ointment. *J Amer Pharm Ass (Prac Ed.)*, 6, 365-366



## B.P. AND B.P.C. OINTMENTS AND BASES

Ointment bases may be divided into four types—

### PROPERTIES

Hydrocarbon bases are not absorbed by the skin, and are, therefore, used when a superficial or simple protective action is desired. The ointments prepared therefrom are either smeared over the skin or applied spread on material.

Hydrocarbon bases are characterised by great stability, they never become rancid, and very few chemicals act upon them. It is, however, impracticable to incorporate more than a small proportion of

### 1 Hydrocarbon Bases

aqueous or alcoholic liquids with hydrocarbon bases alone

### FORMS AND USES

The Pharmacopoeia includes three hydrocarbon bases for use in ointments, namely, Soft Paraffin, Paraffin Ointment and Simple Ointment.

### Soft Paraffin

There are two varieties of this, yellow and white—the latter being the bleached form of the yellow

White soft paraffin is used in ointments containing white or colourless substances, the yellow in others

Soft paraffin (yellow) is used alone as the base in two ointments—

#### DITHRANOL OINTMENT B.P. AND STRONG

#### DITHRANOL OINTMENT B.P.

Dithranol

Yellow Soft Paraffin

These ointments are prepared by trituration and contain 0.1 per cent and 1.0 per cent of dithranol respectively

#### OINTMENT OF AMMONIATED MERCURY AND COAL TAR B.P.C.

Ammoniated Mercury

Coal Tar Solution

Yellow Soft Paraffin

Ammoniated Mercury is a potent and somewhat gritty substance and must be carefully levigated with a small portion of the base before dilution with the

remainder As the Coal Tar Solution is present only to the extent of just over 6 per cent it is readily incorporated in the base to form a stable dispersion although no emulsifying agent is included in the formula

Ointment of Calamine is also prepared with a base of Soft Paraffin

#### Mixed Paraffin Bases

Occasionally, mixtures of paraffins are used to provide bases with a suitable consistency Examples of these are Wool Alcohols Ointment B.P., Emulsifying Ointment B.P., Cetrimide Emulsifying Ointment B.P.C., and Cetomacrogol Emulsifying Ointment B.P.C.

#### Paraffin Ointment B.P.

This is a mixture of hard and soft paraffins with a small proportion of beeswax

Paraffin Ointment is used as the base for Ointment of Boric Acid B.P.C.

### 2 Animal fat Bases

#### PROPERTIES

Animal fat bases are used when the medicament is intended to be absorbed and to have a deeper action These fats resemble the natural fat of the skin and easily permeate it Ointments prepared with an animal fat base are usually applied by friction, which, by forcing the ointment into the skin and increasing local circulation facilitates passage through the epidermis

#### Lard

This is lard which has been specially prepared for pharmaceutical use, being *inter alia* free from water,

alkalis, salt, and rancidity It is very liable to become rancid upon exposure to moisture, light, and air, and it is greasy and unpleasant in use Nevertheless, it is used as the base of Ointment of Gall and Opium B.P.C. More stable substances are now preferred to lard One of these is isopropyl myristate which is used in emollient ointments It is non-greasy and a good solvent for substances commonly applied externally Like lard it has good skin penetrating properties

### 3 Wool Fat and Wool Alcohol Bases

#### Wool Fat (Anhydrous Lanolin)

Chemically, Wool Fat is not a fat but a wax Unlike lard, it does not turn rancid It takes up about 50 per cent of water, and is, consequently, used in ointments in which the proportion of water or other liquid would be too great to allow incorporation with any other fatty base Mixed with soft paraffin it is readily absorbed by the skin and has valuable emollient properties

Wool fat is used as a component in ointment bases, but is too sticky for use alone Its great value is indicated by the fact that it is used in nearly half the B.P. and B.P.C. ointments It is an important constituent of Simple Ointment B.P. and Eye Oint-

ment Base B.P. each of which is used in the preparation of a number of other ointments It is also used in Hamamelis Ointment B.P.C., Hydrocortisone Acetate Ointment B.P., Hydrocortisone Ointment B.P., Ichthammol Ointment B.P.C., and Compound Resorcinol Ointment B.P.C.

#### Lanolin (Hydrous Wool Fat)

This is a mixture of wool fat 70 and Purified Water 30 When mixed with other fats, e.g. lard, soft paraffin, it is able to take up more liquid, and its absorption by the skin is greatly increased by the presence of these fats

Lanolin is used alone as an emollient, and is frequently an ingredient of B.P.C. ointments

**Wool Alcohols**

This is prepared from wool fat, and its properties and uses have already been discussed.

**Wool Alcohols Ointment**

This contains Wool Alcohols, Hard Paraffin, White Soft Paraffin or Yellow Soft Paraffin, and Liquid Paraffin, and forms the subject of Exercise 10 1, when its uses were discussed. The following ointments are prepared with Wool Alcohols Ointment—

Ointment of Salicylic Acid B P  
Oily Cream B P

**Oily Cream**

This is prepared from Wool Alcohols Ointment, and is dealt with in Exercise 10 7. It is used as the base for—

Ointment of Salicylic Acid and Sulphur

**Simple Ointment**

This is a mixture of Wool Fat, Hard Paraffin, and Soft Paraffin. It keeps indefinitely, and cannot go rancid.

Simple Ointment is used as the base in the following ointments—

Ammoniated Mercury Ointment B P	Ointment of Capsicum B P C
Sulphur Ointment B P	
Zinc Ointment B P	

**Base for Eye Ointments**

This is Soft Paraffin (yellow) containing 10 per cent of Wool Fat and 10 per cent of Liquid Paraffin. Before use, the base is filtered to remove all particles of foreign matter, and then sterilised. The eye ointment is then prepared with aseptic precautions, see Chap. 26.

When the strength of an eye ointment is not stated on a prescription, the Pharmacopœia prescribes the following—

**4 Water Miscible Bases****Emulsifying Ointments**

The B P and B.P.C. include three anhydrous emulsifying ointments each capable of admixture with water and aqueous solutions to produce oil in water emulsions. In each case the emulsifying agent is of a different type—anionic, cationic and non ionic—and the ointments are used according to their compatibility with medicaments.

The ointments are—

Atropine Eye Ointment B P	1 0 per cent of Atropine Sulphate
Chloramphenicol Eye Ointment B P C	1·0 per cent of Chloramphenicol
Hydrocortisone Eye Ointment B P C	2 5 per cent of Hydrocortisone Acetate
Hycosine Eye Ointment B P	0 25 per cent of Hycosine Hydrobromide
Mercuric Oxide Eye Ointment B P C	1·0 per cent of Yellow Mercuric Oxide
Phystostigmine Eye Ointment B P C	0 125 per cent of Phystostigmine Sulfate
Synonym Eserine Eye Ointment	
Sulphacetamide Eye Ointment B P	6 0 per cent of Sulphacetamide Sodium

It should be noticed that some of the above contain an alkaloidal salt. These are soluble in water, and it is directed that they shall be dissolved in the minimum amount before incorporation. Water soluble alkaloidal salts, presented in eye ointments in an emulsified state, are rapidly absorbed.

The more important reasons for selecting a mixture of Wool Fat 10 per cent, Liquid Paraffin 10 per cent and Yellow Soft Paraffin 90 per cent as the base for eye ointments are—

1. White soft paraffin is prepared from yellow by bleaching with oxidising agents and acids, followed by washing with water to remove these reagents. Traces of the latter may be present in white soft paraffin, and may give rise to irritation, hence yellow soft paraffin is preferred.

2. Wool fat is included for a number of reasons—

- (a) It ensures perfect emulsification of the aqueous solution of alkaloidal salt incorporated in a number of the eye ointments
- (b) It promotes absorption of alkaloidal salts
- (c) It possesses useful emollient properties

3. Liquid Paraffin 10 per cent is included to give a softer consistency suitable for application to the eyelids

**Emulsifying Ointment B P (Anionic)**

Cetrimide Emulsifying Ointment B P C (Cationic)  
Cetomacrogol Emulsifying Ointment B P C (Non ionic)

Their general formula is—

The emulsifying wax	30%
White Soft Paraffin	50%
Liquid Paraffin	20%

These ointments are used for the preparation of aqueous creams or where easy removal from the skin by washing is desirable. The following are examples.

#### EMULSIFYING OINTMENT B.P.

Aqueous Cream B.P., Buffered Cream B.N.F., Compound Benzoic Acid Ointment B.P.C., Zinc Undecenoate Ointment B.P., Paste of Resorcinol and Sulphur B.P.C.

#### CETOMACROGOL EMULSIFYING OINTMENT B.P.C.

Hydrocortisone Cream B.P.C.

Chlorhexidine Cream B.P.C. Neomycin Cream B.P.C.

#### Macrogol Bases

Polyethylene glycols are a range of water soluble compounds known in this country as Macrogols and having the following general formula—



They are prepared by the polymerisation of ethylene oxide and vary in molecular weight depending on the number of oxyethylene groups in the molecule. The available polyethylene glycols are mixtures and each mixture is known by the average molecular weight. Common examples have average molecular weights of 300 and 400 (viscous liquids) and 1,540 and 4,000 (waxy solids).

Suitable mixtures of such macrogols give products of ointment like consistency. For example Macro-gol Ointment B.P.C. is a mixture of Macrogol 300 and Macrogol 4,000. In the United States of America these compounds have been used to a greater extent.

than in this country and the United States Pharmacopæia includes an ointment of this kind under the name of Polyethylene Glycol Ointment. It contains 40 per cent of macrogol 4,000 and 60 per cent of macrogol 400.

Macrogols 300 and 400 are clear colourless viscous liquids with a faint but characteristic odour. They are miscible with water, alcohol, acetone, benzene and with other glycols but immiscible with ether and the aliphatic hydrocarbons. The number of oxyethylene groups varies in Macrogol 300 from 5 to 5.75 and in Macrogol 400 from 7 to 9.

Macrogol 4,000 is a creamy white solid usually in the form of flakes and almost without odour or taste. It is readily soluble in water, alcohol and chloroform but insoluble in ether.

These mixtures are remarkable in being water-soluble although waxy in appearance. They have good solvent properties and are compatible with most common ointment ingredients such as ammoniated mercury, mercuric oxide, boric acid ichthammol, sulphur and salicylic acid. They must be regarded as incompatible with phenol and phenolic disinfectants as they reduce their bactericidal power.

The activity of many medicaments, however, may be greater in these bases than in the greasy types as the rate of release from macrogol bases is greater.

*Exercises 10.41, 10.42 and 10.43 are examples of ointments with macrogol bases.*

The macrogols are sometimes added to emulsified bases as stabilisers and because of their emollient qualities Exercise 10.43 illustrates the use of a macrogol base containing a medicament in aqueous solution.

#### EXERCISE 10.41

Ammoniated Mercury Macrogol Ointment Make an ointment Label Apply to the affected part Levigate the finely powdered ammoniated mercury with a little of the base, gradually add the remainder with thorough trituration	Send 30 grammes to 100
---	---------------------------

#### EXERCISE 10.42

Salicylic acid Sublimed sulphur Macrogol 400 Macrogol 4,000 Cetostearyl alcohol Make an ointment Label Apply to the scalp as directed. Melt the cetostearyl alcohol with the macrogols and prepare the ointment in exactly the same manner as the previous one or the finely powdered sulphur and salicylic acid may, with advantage, be rubbed down with a little of the macrogol 400 in the first place	0.5 1.5 55.0 37.0 5.0 Send 2 ounces
--	--

**SUGGESTIONS FOR PRIVATE STUDY**

1 Compare the properties of the following—

Lard	Wool fat
Wool Alcohols Ointment	Paraffin Ointment
Cetrimide Emulsifying Ointment	Emulsifying Ointment

2 Suggest a suitable base for—

Eye Ointments
Penicillin Ointment

and give reasons for the choice

3 Devise suitable formulæ and methods for preparing—

- (a) An ointment containing 25% of a hydro-alcoholic liquid.
- (b) An ointment containing 5% of phenol
- (c) A non-staining ointment containing 5% of iodine
- (d) An aqueous cream containing 1% of cetrimide

4 Criticise the following—

(a) A formula for an antiseptic cream—

Euflavine	1%
Emulsifying Ointment	30%
Purified Water	69%

(b) A formula for a non-staining iodine ointment—

Iodine	2 G
Soft Paraffin	38 G

(c) A formula for an eye ointment—

Cocaine	2 G
Soft Paraffin	98 G

(d) A formula for an antiseptic cream—

Cetrimide	1%
Emulsifying Ointment	30%
Distilled Water	to 100%

What modifications or alterations would you suggest?

5 Explain the reasons for the following statements—

- (a) *Re Poulose of Kaolin, Heat at 120°C for 1 hour.*
- (b) *Re Oleated Mercury, 'Heat the mixture to 50°C.'*

6 Why are three different emulsifying ointments included in the B.P. and B.P.C.

7 Comment critically on the following statements—

(a) Under Starch Glycerin B.P.

'Heat gently with constant stirring until a translucent jelly is formed.'

(b) Under Oleated Mercury B.P.C.

'heat at 50°C, with occasional stirring until combination is effected.'

## PLASTERS

Plasters, like ointments, are solid preparations containing a medicament for application to the skin. They differ from ointments in that the medicated mass is spread on cloth or other material. Plasters may be in contact with the skin for protracted periods. Those in common use today are of the 'pressure sensitive' or 'self-adhesive type'. They have a rubber basis which may contain a medicament. The rubber is brought into suitable form by plasticisers which assist the spreading of the mass and tackifiers which give it adhesiveness. The preparation of these is, of course, a manufacturing

process. The older type of plaster, not now official, generally had a basis of lead plaster and was hard at ordinary temperatures. When applied to the body it became soft, adhesive and flexible, but did not melt. In preparing plasters of this type the mass was melted and spread upon suitable material with a plaster iron.

Today plasters are not made extemporaneously and for details of the manufactured types in common use the student is referred to the B.P.C. and *Tutorial Pharmacy*.

## Suppositories, Pessaries, Urethral and Nasal Bougies

ALL these consist of a base uniformly medicated with some substance they differ chiefly in size and shape

### BASES

It is important that these preparations should retain their moulded shape until body temperature is reached, when they should melt or soften and release the medicament *in situ*

Until recently the bases most commonly used were Theobroma Oil and glycerol-gelatin. Today a number of new bases are available which possess advantages over these. The British Pharmaceutical Codex 1959 permits the use of other bases in place of Theobroma Oil provided that the melting point of the suppositories is not above 37°C. In general suppository bases may be divided into three types

- 1 Oily Bases
- 2 Aqueous Bases
- 3 Emulsifying Bases

The ideal base should have the following properties—

- 1 It should melt at body temperature or dissolve or disperse in body fluids
- 2 It should release any medicament readily
- 3 It should keep its shape when being handled
- 4 It should be non toxic and non irritant to the mucous membrane
- 5 It should be stable on storage
- 6 It should be compatible with any added medicament
- 7 It should be stable if heated above its melting point
- 8 It should be easily moulded and should not adhere to the mould
- 9 It should be mouldable by pouring or cold compression

### 1 Oily Bases

#### THEOBROMA OIL (Syn Cocoa-Butter)

This is a mixture of the glyceryl esters of stearic, palmitic, oleic and other acids. It conforms to most of the above requirements of an ideal base. Its main disadvantages are that overheating alters its physical characteristics and that it has a tendency to adhere to the mould when solidified. When melted and cooled theobroma oil solidifies in different crystalline forms depending on the temperature of melting, the rate of cooling and the size of the mass. Overheating is likely to produce on cooling unstable gamma crystals which melt at about 15°C, or alpha crystals which melt at about 20°C. If melted at 35° to 36°C and slowly cooled it forms stable beta crystals. All forms return eventually to the stable condition but this may take several days. It will be seen, therefore, that extreme care must be taken in dispensing to ensure rapid solidification to the stable condition which has a melting point just below body temperature.

Adherence to the mould may be prevented by the use of a suitable lubricant the use of which is described later.

Substances which dissolve in theobroma oil lower its melting point and may do so to such an extent that the product would be too soft for use. Added beeswax may be used to restore the melting point but suppositories made with this addition may, on storage, fail to melt at body temperature, according to Soulsby and Hopkins (1956).

For the incorporation in oil of theobroma suppositories of aqueous solutions lactic acid, phenol and ichthammol, Rae (1942) found that the addition of 10 per cent of Lanette Wax SX was suitable.

**HYDROGENATED OILS**

The use of these as suppository bases has been investigated by Caldwell (1939). He recommended the use of hydrogenated palm kernel oil, especially for use in tropical countries.

**2. Aqueous Bases****GLYCERO GELATIN**

This is a mixture of glycerin and water made into a stiff jelly by the addition of gelatin—the proportion being varied according to the purpose for which the preparation is intended.

Glycerin Suppository Mass B.P., as used for Plain Glycerin Suppositories, is also suitable for many medicated forms.

Gelato-Glycerin B.P.C. 1954, which is much firmer, is suitable for nasal and urethral bougies.

Gelatin may be obtained in translucent sheets, shreds, granules or powder. Good quality powdered gelatin is most satisfactory for dispensing purposes as many samples of sheet gelatin obtainable today are difficult to dissolve.

**MACROGOL BASES**

The following mixtures of polyethylene glycols (Macrogols, Carbowaxes) of varying hardness have been described by Hasler and Sperandio (1953) for use as suppository bases. Like glycerine gelatin bases they are miscible with body fluids.

1 Macrogol '4000'	33%
Macrogol '6000'	47%
Water	20%
2 Macrogol '1540'	33%
Macrogol '6000'	47%
Water	20%
3 Macrogol '1540'	33%
Macrogol '6000'	47%
Macrogol '400'	20%

The dispersibility of these bases is indicated by the fact that they dissolve in water at 37°C in 25 to 30

minutes. Macrogols are incompatible with phenols and reduce the antiseptic effect of quaternary ammonium compounds.

**3 Emulsifying Bases**

A number of proprietary bases of excellent quality is available of which the following are representative \*

**MASSA ESTERINUM (Adeps Solidus—German Pharmacopoeia 6th Edition, 3rd Supplement)**

This is a mixture of the monoglycerides, diglycerides and triglycerides of the saturated fatty acids having the formulae  $C_{11}H_{22}COOH$  to  $C_{11}H_{22}COOH$ . Several grades are available.

**WITEPSOL (Formerly called Imhausen Bases)**

These consist of hydrogenated triglycerides of lauric acid with added monoglycerides. Nine grades are available.

**MASSUPPOL**

This consists of glyceryl esters, mainly of lauric acid, to which a very small amount of glyceryl monostearate has been added. A general-purpose base which satisfactorily meets most requirements.

In the cases of Massa Esterinum and Witepsol the various grades are formulated to meet different requirements such as, absorption of considerable quantities of liquid, the incorporation of substances which lower the melting point, for conditions where raised temperatures may be encountered during transport and storage, and for cold compression manufacture.

All these compounds have advantages over cocoa butter in that the physical characteristics are not altered by overheating and that they do not adhere to the mould, which needs no lubricant. Indeed, lubrication is a disadvantage as it may spoil the glossy appearance of the product.

They solidify rapidly and as they all contain an emulsifying agent they can absorb fairly high percentages of aqueous liquids. The emulsifying agents

	Manufactured by	Available from
* <i>Massa Esterinum</i>	Edelfette Werner Schluter, Hamburg Eidelstedt, Germany	R. W. Greef and Co., Ltd., 31/45 Gresham Street, London, W.C.2
<i>Witepsol</i>	Chemische Werke Witten, G.m.b.H., Ruhr Germany	Chemicals Trading Co., Ltd., Cree House 18/20 Creechurch Lane, London, E.C.3
<i>Massuppol</i>	Crok and Laan, Wormerveer Holland	A. Goldrei and Co., Ltd., 50, Mark Lane, London, E.C.3

are monoglycerides which form water-in-oil emulsions, and this would seem more rational than the use of oil-in-water emulsifying waxes which are sometimes advocated.

They are white odourless waxy solids and are non-irritant. The appearance of suppositories made with them is usually excellent.

#### Absorption of Medicament

Suppositories may be used to obtain a local or systemic effect. Local action may be produced by antibacterial substances, analgesics, emollients, astringents, etc. For a systemic effect the drug must be absorbed by the mucosa. The rate of absorption depends on several factors, and much investigation still requires to be carried out before the picture is clear. The following references give some idea of the difficulty of arriving at any simple assessment of the problem.

Charannat and colleagues (1949), using rabbits, tried suppositories containing methyl nicotinate and measured the rise in temperature by means of a thermocouple attached to the animal's ear. They found that cocoa butter gave a quicker release of medicament than a macrogol base but the effect lasted longer in the latter case.

Allawala and Riegelman (1953) claim that surface-active agents affect the rate of release of a drug from the base, sometimes increasing and sometimes slowing it down.

Cacchillo and Hassler (1954) found that, using acetosalicylic acid as medicament a macrogol base was superior to cocoa butter or glycer-gelatin in the speed of releasing the drug.

Gradnik (1955) stated that in general fatty media are used when a slow prolonged absorption is required.

Whitworth and Larocca (1959) used sodium pentobarbitone as medicament and found that bases containing surface-active agents such as Tweens, gave the fastest rate of release.

Pennati and Steiger-Trippi (1958) found that the absorption of sulphasomidine (Elkosin Ciba) was quicker from a cocoa butter base than from glycer-gelatin, macrogol or emulsifying bases.

#### SUPPOSITORIES (*Suppositoria*)

These are intended for introduction into the rectum, and are commonly made in either of two shapes—

- (a) Cone-shaped, with rounded apex, weighing about 15 grains or 1 gramme
- (b) Torpedo-shaped, weighing about 30 grains or 2 grammes. This shape gradually widens from

the base for about three-quarters of its length, and then tapers off to a blunt apex. It has the advantage that when the widest part has been inserted, the sphincter muscle presses the suppository onwards into the rectum. Furthermore, the size allows for incorporation of a larger quantity of medicament.

#### PESSARIES (*Pessi*)

These are intended for introduction into the vagina, and are made cone-shaped or wedge-shaped, with a rounded apex. There are two sizes, 60 and 120 grain.

#### URETHRAL BOUGIES (*Cereoli*)

These are intended for the urethra, and are 2½ in long (weighing about 15 grains). Cocoa-butter is used as the base.

#### NASAL BOUGIES (*Bugunara*)

These are similar in shape to urethral bougies, but thinner and not more than 3½ in. in length, weighing about 18 grains. They are always made with a gelato-glycerin base.

#### EAR CONES (*Aurinaria*)

These are very rarely required, and, in the absence of a special mould, they may be prepared in a urethral bougie mould and cut to the required size. The medicament is usually prescribed as a percentage, and the actual weight of the cone is unimportant as regards the quantity of contained medicament. Unless otherwise prescribed, they are made with a cocoa butter base.

#### Containers for Suppositories, etc

The above products are sent out in partitioned boxes. If these are not available a powder-box may be used and each product separated from its neighbour by waxed paper folded to shape. It should not be necessary to label suppositories and pessaries with the words 'Not to be taken' but if the directions are not explanatory the words 'For rectal use' or 'For vaginal use' may be added and 'Keep in a cool place'.

#### MOULDS

These are made to numerous standard shapes, and the metal is sometimes plated to prevent interaction with medicaments and to give the suppository a better finish—consequently, the interior of the moulds should never be scraped. For cleaning, they should be immersed in hot water, and the cavities wiped with a piece of gauze. The cleaned mould should then be immersed in cold water to dissipate the contained heat.

*Calibration of Moulds*

Unless moulds are available in both Metric and Imperial systems, a 15 grain mould may be regarded as equal to a 1-gramme mould, and so on. Calibration with the base is, however, necessary for all moulds, and having once determined the exact capacity of a particular mould the figure should be used in calculating the quantities. For simplicity of calculation in the following exercises the nominal capacity of the mould has been regarded as correct, but the figures must be adjusted, when necessary, according to the calibration figure.

**DISPLACEMENT VALUE OF SOLID MEDICAMENTS**

The volume of a suppository from a particular mould is obviously uniform, but its weight will vary according to the density of medicaments, as compared with cocoa-butter with which the mould was calibrated. Consequently, products made from moulds cannot be prepared accurately unless allowance is made for the alteration in density of the mass due to added medicaments. The quantity of medicament which displaces 1 part of cocoa-butter (called the displacement value) is the most convenient method of making this allowance.

Table 111

Alum	2.0	Lead Iodide	5.0
Aminophyllin	1.5	Mercury Ointment	1.5
Bismuth Oxynitrate	5.0	Morphine Hydrochloride	1.5
Bismuth Subgallate	2.5	Opium (powdered)	1.5
Boric Acid	1.5	Peptone	1.5
Chloral Hydrate	1.5	Phenol	1.0
Cinchocaine	1.5	Quinine Hydrochloride	1.0
Cocaine Hydrochloride	1.5	Resorcinol	1.5
Hamamelis Dry Extract	1.5	Salol	1.5
Hydrocortisone	1.5	Silver Proteinate	1.5
Hydrocortisone Acetate	1.5	Tannic Acid	1.0
Ichthammol	1.0	Zinc Oxide	5.0
Iodoform	4.0	Zinc Sulphate	2.0
Lead Acetate	3.0		

**EXAMPLE 111**

The displacement value of Iodoform is 4, i.e. 1 grain of cocoa-butter is displaced by 4 grains of Iodoform.

Twelve suppositories, each containing 3 grains of Iodoform, are to be made.

The total quantity of medicament is  $12 \times 3 = 36$  grains, and this displaces  $\frac{36}{4} = 9$  grains of cocoa-butter. Therefore the quantity of base required (using a 15-grain mould) is  $(12 \times 15) - 9 = 171$  grains.

The total weight of the 12 suppositories will therefore be  $171 + 36 = 207$  grains, i.e. rather more than 17 grains each. Hence, although made in a 15-grain mould, the suppositories weigh over 17 grains. Using standard moulds, it is impracticable to make suppositories each containing 3 grains of Iodoform and weighing 15 grains. Hence, in prescriptions and formulae, 15 grains indicates the standard size of mould, and the dispenser is left to make necessary corrections for the displacement value of the medicament.

Table 111 shows the displacement value for substances prescribed in suppositories, pessaries, and bougies referred to cocoa-butter—

Displacement values may be determined as follows—

Prepare and weigh six suppositories of Theobroma Oil (or other base) =  $a$  grains

(continued overleaf)

*Example 11.1 continued*

Prepare and weigh six suppositories containing, say 40 per cent of medicament =  $b$  grains

Calculate the amount of Theobroma Oil,  $c$  grains and medicament,  $d$  grains in the six suppositories

Now,  $a - c$  grains = the weight of Theobroma Oil displaced by  $d$  grains of medicament

$$\text{Displacement value of the medicament} = \frac{d}{a - c}$$

**EXAMPLE 11.2**

Weight of six unmedicated suppositories = 90 gr

Weight of six suppositories containing

40% of zinc oxide = 132 gr

Theobroma Oil in this =  $\frac{60}{100} \times 132 = 79.2$  gr

Zinc oxide content =  $\frac{40}{100} \times 132 = 52.8$  gr

Theobroma Oil displaced by 52.8 grains of zinc oxide =  $90 - 79.2 = 10.8$  gr

Therefore, the displacement value of zinc oxide =  $\frac{52.8}{10.8} = 5$  (approx)

Such determinations often produce variable results because more or less air becomes entrained in the mass during stirring

For substances not included in Table 11.1 the following method may be used—

Subtract the full weight of all the medicaments from the weight of cocoa butter required to fill the mould and, when set, trim down the flat surface of the suppository until its weight is that of the cocoa butter required to fill the mould used.

### DISPLACEMENT VALUE OF LIQUID MEDICAMENTS

The displacement value of liquids may be taken as 1 without incurring serious error

#### Lubrication of Moulds

Lubricating the cavities of the mould is helpful\* in producing elegant cocoa butter suppositories free from surface depressions

The lubricant must be different in nature from the suppository mass, otherwise it will become absorbed, and fail to provide a buffer film between the mass and the metal. Consequently, an oily lubricant is useless for cocoa butter suppositories for these the follow-

ing has been found satisfactory—

Soft Soap	of each	1 part
Glycerin	of each	5 parts
Alcohol (90%)		

This lubricant would, for the reason noted above, be quite unsuitable for use with a gelato-glycerin mass, and for this it is necessary to use an oil, e.g. liquid paraffin, olive, or almond oil.

The lubricant should be applied on a pledge of gauze or with a small fairly stiff, brush

### CLASSIFICATION

The exercises in this chapter will be based upon the following classification—

Class 1 Cocoa-butter Suppositories containing Insoluble Solids

Class 2 Cocoa-butter Suppositories containing Soluble Solids

Class 3 Cocoa butter Suppositories containing Semi-solids

Class 4 Cocoa butter Suppositories containing Liquids

Class 5 Glycerin Suppositories

Class 6 Soap Glycerin Suppositories

\* Lubrication is undesirable when the mass contains alcohol, or fluorescent substances e.g. acriflavine proflavine

**Class 1. Suppositories Containing Insoluble Solids General Method****STAGE 1**

Thoroughly lubricate the mould, and invert it on ice to drain and cool Unnecessary with new bases

**STAGE 2**

Place in a dish the weighed amount of shredded cocoa butter stand it over a warm-water bath until two thirds melted, and then remove it The remainder will melt with stirring, and this course will prevent over-heating the base, which often causes unsatisfactory suppositories

**STAGE 3**

Place on a warmed slab the weighed quantity of powdered medicament, and over it pour about half the melted base Work into a smooth cream as quickly as possible, using a flexible spatula, transfer to the dish, and stir to form a homogeneous mixture

Cool the mixture on cold water until it is just too thick to pour and then return it to the water bath for

a few seconds, stirring until the mass becomes pourable Fill each cavity to overflowing taking care to stir the mass continuously to ensure even distribution of the medicament As cocoa butter contracts upon cooling, overfilling is necessary to prevent hollows in the tops of the finished suppositories When the liquid has just set, \* this excess is trimmed off horizontally, using a sharp knife

**STAGE 4**

Stand the mould for half an hour in a cold place or on ice, if available Open the mould, remove the suppositories, and lightly wipe off any lubricant with a clean cloth Provided the mould was properly lubricated and the melted mass not too hot, the products should be detached without the least difficulty

\* Trimming at this stage produces a clean cut which cannot be obtained when the mass has become hard.

**EXERCISE 11 1**

Mitte Suppositoria Bismuthi Subgallatis, sex

Signentur Unum quotidie utendum

The B P C directs that suppositories containing 300 mg shall be supplied on unspecified orders

*Calculation—*

An excess of material must always be provided, because of unavoidable losses during preparation To cover this, when using a 1 G or 2 G mould, prepare for 2 suppositories above requirements Using a 1 G mould, and calculating for 8, the quantities will be—

Bismuth Subgallate	2 4 G
This will displace $\frac{2}{5}$	= 0 96 G
Cocoa butter, 8 — 0 96	= 7 04 G

Follow the *General Method* described above

**EXERCISE 11 2**

*Recipe—*

Acidi Borici gr x

Cacaonis Butyri, quantum sufficiat ut fiat pessus

Mitte tales quinque Signa Unus omni nocte utendum

*Calculation*

For 6 x 120 grain pessaries the quantities will be—

Boric Acid	60 grains
------------	-----------

This will displace $\frac{60}{15}$	= 40 gr
------------------------------------	---------

Cocoa butter, 720 — 40 = 680 gr	680 grains
---------------------------------	------------

Follow the *General Method*, above

**EXERCISE 11.3***Recipe—*

Ext Hamam Sicc gr ij  
Olei Theobromatis quantitatem sufficientem ut fiat suppos  
itorum grana triginta

Mitte talia sex Signentur Unum si opus sit utendum

*Calculation*

For 8 torpedo shaped suppositories the quantities will be—

Hamamelis Dry Extract 16 grains

This will displace  $\frac{16}{15}$  = 10½ gr

Cocoa butter, 240 — 10½ = 229½ grains

Follow the *General Method*, p. 171

**EXERCISE 11.4***Recipe—*

Ext Hamam. Sicc. gr iss

Zinc Oxid. gr v

OI Theobrom q s

Fiat suppositorium. Mitte vi

Signa—Unum nocte si opus sit utendum

To produce a homogeneous suppository of uniform composition and colour in this case it is advisable to use a small quantity of water as a levigating agent Three minims of water per suppository can be readily incorporated

*Calculation*

For 8 × 15 grain suppositories the quantities will be—

Hamamelis Dry Extract . 12 grains

This will displace  $\frac{12}{15}$  = 8 grains of oil of theobroma

Zinc Oxide 40 grains

This will displace  $\frac{40}{5}$  = 8 grains of oil of theobroma

Water 24 minims

This will displace approximately 22 grains of oil of theobroma

The quantities of oil of theobroma, therefore, will be

$$8 \times 15 - (8 + 8 + 22) = 82 \text{ grains}$$

Levigate the zinc oxide and the dry extract with the water using a vulcanite spatula on an ointment tile until quite homogeneous Gradually add the melted oil of theobroma and proceed according to the *General Method* on p. 171

**Filling Bougie Moulds**

In filling Bougie moulds the mass is not likely to reach the bottom of the narrow cavities, and to overcome this difficulty either of the following methods may be used, the first being preferable—

- (a) Adjust the two halves of the mould to form a V just held together by the first thread of the screw, and close the sides and bottom with a narrow strip of paper smeared with Soft Paraffin. Cool the mass until it is just pourable and then pour

it into the open mould, chiefly in the centre. Then press the base of the mould, thereby forcing the mass upwards, then screw the mould up tightly.

- (b) Lay the mould open, fill one side with the mass, which should be only just pourable. Quickly superpose the empty half of the mould, stand it

upright, and fill the cavities with more of the mass in the ordinary way.

Both methods require a larger excess of material than needed for filling suppository moulds, and it is preferable to weigh quantities for 10 bougies in order to produce 6.

### EXERCISE 115

#### *Recipe—*

Argenti Proteinatis gr iss

Olei Theobromatis quantum sufficit ut fiat cereolus

Mitte tales sex. Signa More dicto utendus

#### *Calculation*

For 10 bougies—

Silver Proteinate 15 grains

This will displace  $\frac{15}{15} = 10$  gr

Cocoa butter, 150-10 = 140 grains

Follow the *General Method*, p 171, adopting method (a) given above for filling

### EXERCISE 116

Prepare 6 × 30-grain suppositories (torpedo-shaped) containing 20 per cent Ichthammol. Use Macrogol base No 3 see p 167

#### *Calculation*

Notice that in this prescription the finished suppositories must contain 20 per cent of their weight of Ichthammol, hence consideration of its displacement value does not arise. Each suppository will weigh about 30 grains, and 240 grains should be prepared to cover loss—

Ichthammol 48 grains

Macrogol base 192 grains

Follow the *General Method* p 171

### Class 2 Cocoa butter Suppositories Containing Soluble Solids

The melting point of cocoa butter is lowered when substances are dissolved therein. In some cases the reduction is only slight, and the finished suppositories are quite firm at body temperature; in others the suppositories would be too soft for use unless the melting point were raised. The BPC sanctions the addition of beeswax to suppositories when necessary, owing to the above or other causes, but it stipulates that the melting point of the mixture must not exceed 37°C, i.e. must remain approximately 1°C below body temperature.

Substances, soluble in cocoa butter, likely to be prescribed in suppositories are—

Phenol Chloral Hydrate

The melting point of beeswax is 62–64°C, that of cocoa butter 30–35°C. In preparing an admixture it is evident, therefore, that the cocoa butter must be raised to approximately 60°C, i.e. 15–20°C above its melting point. This treatment temporarily lowers its melting point, thus delaying setting and giving no immediate advantage. The addition of beeswax should be avoided by the following method—

### METHOD FOR SOLUBLE SOLIDS

Method suitable for Phenol up to about 15 per cent of suppository, and Chloral Hydrate up to about 25 per cent

## STAGE 1

As Stage 1 of *General Method*, p 171

## STAGE 2

As Stage 2 of *General Method*, but cool and stir the cocoa butter to the consistency of thick cream.

## STAGE 3

Add the weighed quantity of powdered medicament to the cocoa butter, stir until it dissolves, and complete the succeeding stages as before

N B In such cases it is more satisfactory to use one of the modern bases mentioned on p 167

## EXERCISE 11.7

Prepare six suppositories each having the following content—

Chloral Hydrate	gr iii
Cocoa butter	q s

Label The Suppositories Use as directed

Follow the *Method for Soluble Solids* described on p 173

## Class 3 Cocoa butter Suppositories containing Semi-solids

It has already been mentioned that stable mechanical emulsions may be prepared with solid fats. This fact is utilised in preparing suppositories containing aqueous or hydro-alcoholic liquids. It requires more care, however, to make a mechanical emulsion with an aqueous or hydro-alcoholic liquid and cocoa butter than with a fat with the latter stirring may be continued during passage from liquid to solid state, but with cocoa butter stirring must cease before the liquid becomes unpourable.

Two points to be watched are—

- (a) The formation of a very perfect emulsion by vigorous stirring of the fluid mixture
- (b) Pouring at the last possible moment, i.e. when the globules of the distributed aqueous liquid are enclosed in a solidifying film of cocoa butter

## METHOD FOR SEMI SOLIDS

## STAGE 1

As Stage 1 of the *General Method*, p 171

## STAGE 2

As Stage 2 of the *General Method* (p 171) placing in the dish a quantity of cocoa butter slightly greater than the nominal capacity of the moulds

## STAGE 3

Weigh out the prescribed quantity of semi-solid place it on a warmed slab and triturate with sufficient water to form a homogeneous liquid of the consistency of glycerin, or rather thinner. Transfer to a tared dish and add sufficient of the melted cocoa butter to produce a weight equal to the nominal capacity of the moulds, i.e. their capacity for plain cocoa butter. Stir vigorously to form a mechanical emulsion, and complete in the usual manner.

If soluble or insoluble substances, or both, are present, the methods are combined

N B Provided Theobroma Oil is not specifically prescribed one of the emulsifying bases mentioned on p 167 is completely satisfactory

## EXERCISE 11.8

## Recipe—

Extracti Belladonnæ Viridis (B P C 1954) gr ii

Olei Theobromatis quantum sufficiat ut fiat suppositorium grana quindecim

Mitte talia sex. More solito utendum.

Follow the *Method for Semi solids* weigh for 8, and thin down the extract with water

## EXERCISE 11.9

## Recipe—

Ichthammolis gr x

Olei Theobromatis quantitatem sufficientem ut fiat pessus grana sexaginta

Mitte tales quinque Signetur Unus omne nocte adhibendus

Follow the *Method for Semi solids*, above Be especially careful to use as low a temperature as possible in making this suppository

## Class 4 Cocoa butter Suppositories containing Liquids

The commonly prescribed liquids fall into two groups—

- Liquids which are volatile (e.g. Eucalyptus Oil) or which owe their medicinal value wholly to volatile constituents (e.g. Balsam of Peru). With these it is evident there must be no evaporation.
- Liquids which owe their medicinal value to non volatile constituents. With these removal of part of the solvent is immaterial.

Proportion of Liquid which can be Incorporated with Cocoa butter

The addition of an oily liquid to cocoa butter lowers its melting point and it is evident that if the proportion is large the mixture will be a soft paste. No precise rule can be given regarding the proportion of oily liquid which can be incorporated with cocoa butter without producing a mixture too soft for

suppositories because the range of melting point of cocoa butter itself is wide (30–35°C) and different liquids have different effects. In general the maximum is about 10–15 per cent—above this beeswax must be added.

As much as 20 per cent of an aqueous or alcoholic liquid can be incorporated in cocoa butter as a mechanical emulsion. This proportion rarely occurs in prescriptions but when it is exceeded the liquid must be concentrated by evaporation (if permissible) to form less than 20 per cent of the product. The methods of incorporating liquids are illustrated in Exercises 11 10 to 11 14.

If 15 grains is insufficient to absorb a prescribed quantity of liquid a 30-grain suppository may be made. The new suppository bases mentioned on p. 167 are superior to Theobroma Oil for this purpose since they contain emulsifying agents.

## EXERCISE 11 10

## Recipe—

Olei Eucalypti	<i>m</i> x
Olei Theobromatis quantum sufficiat ut fiat pessus grana sexaginta	

Mitte tales sex Signa Unus hora somni utendus

## Method

Complete Stages 1 and 2 of the *General Method* p. 171, add the Oil and then proceed in the usual manner.

## EXERCISE 11 11

## Recipe—

Ext Bellad Liq	0 15 ml
Ol Theobrom	q s

Fiat suppositorium Mitte sex

Signa Unum nocte moneque utendum

## Method

Complete Stages 1 and 2 of the *General Method* p. 171, add the Extract and stir vigorously to form a mechanical emulsion. Complete as usual.

## EXERCISE 11 12

## Recipe—

Extracti Hamamelidis Liquidi	<i>m</i> xv
Olei Theobromatis quantum sufficiat ut fiat suppositorium grana triginta	

Mitte talia sex Signa Modo dicto utendum

## Calculation and Method

The suppositories as prescribed, contain 50 per cent of liquid. The medicinal value of the liquid extract is due to its non volatile constituents hence it may be evaporated. It is evident that the displacement should be calculated on the volume of evaporated liquid.

(continued overleaf)

## Exercise 11.12 continued

Place 120 minims of the liquid extract in a tared dish, evaporate over a water-bath to about half the volume, cool *thoroughly*, mix to form a homogeneous liquid (i.e. re-dissolve any extractive matter which may have separated on the side of the dish), and then add sufficient melted cocoa butter to produce 240 grains. Stir vigorously to form a mechanical emulsion, and complete as usual.

The addition of 1 grain of cetostearyl alcohol per suppository to the evaporating liquid assists in the subsequent mixing and helps to prevent solid matter adhering to the dish.

## EXERCISE 11.13

## Recipe—

Adrenalinæ	gr 1/400
Cocaine Hydrochloridi	gr $\frac{1}{4}$
Olei Theobromatis	quantum sufficiat ut fiat suppositorium grana xxx

Mitte talia sex Signentur Si opus sit utendum

## Calculation

As mentioned under Mixtures (p. 49) the pharmacopelial solutions are often convenient for small quantities of potent substances, and thus, in the present instance, Adrenaline Solution will be used. The official solution contains 1 grain of Adrenaline in 1,100 minims, therefore 1/50 grain, the quantity needed for 8 suppositories, will require 22 minims. This volume represents less than 10 per cent of the suppository, hence evaporation is unnecessary.

Cocaine Hydrochloride is soluble in water and will therefore dissolve in the solution. The quantity required, 2 grains, will increase the volume of the solution to approximately 23 minims.

The working formula is therefore—

Adrenaline Solution	22 minims
Cocaine Hydrochloride	2 grains
Cocoa butter	217 grains

## Method

As for Exercise 11.11 (q.v.)

## EXERCISE 11.14

## Recipe—

Iodoformi	
Balsami Peruviani	. ana gr iii
Olei Theobromatis	quantitatem sufficientem ut fiat suppositorium grana triginta.

Mitte talia quinque Signentur Unum more dicto utendum.

## Calculation

Castor Oil (50%) must be added to Balsam of Peru to render it miscible with cocoa butter. The displacement value of Iodoform is 4, 24 grains will therefore displace 6 grains of cocoa butter. The liquids may be taken as displacing their own weight of cocoa butter. The weight of cocoa butter required, calculating for 8 suppositories will be

$$8 \times 30 - (6 + 24 + 12) = 198 \text{ grains.}$$

The working formula is, therefore—

Balsam of Peru	24 grains
Castor Oil	12 grains
Iodoform	24 grains
Cocoa Butter	198 grains

*Method*

Follow the *General Method*, p. 171, mixing the balsam and oil on the slab with a portion of the melted cocoa butter, and then incorporate the iodoform in the usual manner

**Class 5 Glycerin Suppositories**

Gelatin dissolves in hot water, forming a solution which sets to a jelly. This fact is used to convert glycerin into solid form for use as a suppository, e.g. Glycerin Suppository B.P.

A suitable gelatin jelly is also used as a base for other medicaments besides glycerin. The two gelatin bases used are—

**1 Glycerin Suppository B.P., i.e. mass**

This is a suitable base for medicated suppositories and pessaries containing solid medicaments, or not more than about 20 per cent of semi solid or liquid medicaments—with more than this the mass becomes too soft.

**2 Gelato-glycerin B.P.C. 1954**

This is a stiffer mass and is, therefore, used for suppositories and pessaries containing 20 per cent or more of a semi solid or liquid medicament, it is also necessary for nasal and urethral bougies.

A gelatin base is incompatible with many of the substances prescribed in suppositories, e.g. Tannic Acid, Ferric Chloride, Gallic Acid, and for this and other reasons it is less frequently used than cocoa butter. Glycerin suppositories containing Ichthammol become insoluble on storage.

**Correction for Capacity of Mould with  
Glycerin Suppository Base**

Glycerin Suppository B.P. contains 70 per cent w/w of glycerin, and it follows that its specific gravity is

much higher than that of cocoa butter. As the last-named is used for standardising the capacity of moulds, a correction is necessary when using a gelatin base.

The specific gravity of cocoa butter is practically 1.00 that of Glycerin Suppository 1.20 and Gelato-glycerin 1.15, and these figures must be used in calculating the quantity of gelatin base required. The methods and calculations for displacement value are those already described for cocoa butter suppositories—the quantity of cocoa butter which would be used if it were the base, multiplied by 1.20 or 1.15, giving the required quantity of Glycerin Suppository mass or Gelato-glycerin respectively.

The formula and directions for making glycerin suppository mass are given in the B.P. The mass is poured into moulds holding 1, 2, or 4 grammes. As mentioned, glycerin suppository mass has a specific gravity about 1.2, so that it would be necessary to have special moulds for this mass. Moulds holding the above quantities of cocoa butter would obviously hold 20 per cent too much. In practice the B.N.F. directions are interpreted to mean a cocoa butter capacity of 1, 2, or 4 grammes, and the sizes are conveniently described as follows—

1 G (15 grain)	Infant's size
2 G (30 grain)	Medium size
4 G (60 grain)	Large size

**EXERCISE 11.15**

**Send 6 Glycerin Suppositories, Large size**

Preparing for 7, the quantity of mass will be  $7 \times 4 \times 1.2 = 33.6$  G, therefore prepare 40 G.

After solution of the gelatin, stirring should be avoided, as it gives rise to air bubbles, which detract from the appearance of the suppository. It is preferable to just fill the cavities because the mass does not contract upon cooling, but if the cavities are inadvertently overfilled the excess may be trimmed off (after setting) with a hot sharp knife.

## Class 6 Soap Glycerin Suppositories

Stearin soap (i.e. curd soap sodium stearate) has certain advantages over gelatin for making glycerin sufficiently hard for suppositories—

- A larger quantity of glycerin can be incorporated—actually up to 95 per cent of the mass
- Soap assists the action of the glycerin whereas gelatin does not

The disadvantage is that soap glycerin suppositories are very hygroscopic, and require to be wrapped in waxed paper or pure tin foil and protected from the atmosphere.

The soap is used *per se*, or formed in glycerin solution by interaction between sodium carbonate and stearic acid.

## Suppositories made by Compression

Suppositories are also made by hand or power-operated compressing machines. In these the pre-

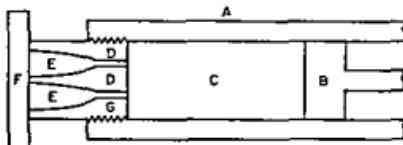


Fig 111 DIAGRAM OF COMPRESSING MACHINE FOR SUPPOSITORIES

pared mass C is placed in a cylinder A and forced through a narrow opening D by means of a piston B into a mould. The threads of mass passing into the mould G are compressed until a homogeneous fused mass is formed in E. On the removal of the retaining stop plate F the suppositories are ejected by further pressure. The operation of the machine is shown diagrammatically in Fig 111. Moulds are of different sizes and contain several cavities.

## EXERCISE 1116

Prepare 6 × 30 grain (torpedo-shape) Soap Glycerin Suppositories B.P.C.  
1949

## Formula—

Glycerin	90 G
Sodium Carbonate	45 G
Stearic Acid	75 G

## Calculation

The specific gravity of this mass is about 1.25 so that each suppository will weigh about 39 grains. Therefore prepare 400 grains of mass to cover loss in skimming and to allow for the generous overfilling necessary with this mass.

Dissolve the sodium carbonate in the glycerin with the aid of gentle heat, add the stearic acid and heat carefully until effervescence ceases and solution is complete. Skim the surface and pour the solution when it is quite hot and free from bubbles, overfill the moulds because considerable contraction occurs and trim level when set. The process may be hastened by heating the mixture over a bunsen until the reaction starts then transferring the dish to a water bath.

## SUPPOSITORIES AND PESSARIES OF THE B.P., B.P.C., AND B.N.F.

The quantities given below are those which must be dispensed in each suppository or pessary when the following are ordered without a specified strength

## Suppositories

B.P.

Glycerin Suppositories (see p. 177)

## B.P.C.

Aminophylline 360 mg

(This suppository is made in a 2 G/mould size)

Suppositories of Bismuth Subgallate 300 mg

Suppositories of Bismuth Subgallate,

Compound Bismuth Subgallate 200 mg with

Resorcinol 60 mg

Zinc Oxide 120 mg

Peru Balsam 60 mg

Suppositories of Cinchocaine	10 mg	Pessaries	
Suppositories of Hamamelis		B P C	Mould size
Suppositories of Hamamelis and Zinc Oxide	200 mg of Dry Extract 200 mg Dry Extract with Zinc Oxide 600 mg	Pessaries of Crystal Violet Pessaries of Ichthammol Pessaries of Lactic Acid Stilboestrol (Propylene glycol as solvent)	0.5% 4 G 5.0% 8 G 5.0% 8 G 0.5 mg 4 G
(This suppository is made in a 2 G/30 gr mould size)			
Suppositories of Morphine	15 mg Morphine Hydrochloride	B N F	
Hydrocortisone	25 mg	Piroflavine Pessaries	0.2% 8 G/120 gr
A 1 G/15 gr mould size is to be used except where otherwise indicated		All these pessaries are prepared with Glycerin Suppository Base (Pessaries of Acetarsol B P C are made by compression using the Moist Granulation Process)	
If Oil of Theobroma is not specified, other suitable bases may be used (see p 167)			

**REVISION EXERCISES** 1 Habeat Suppositoria Aminophyllinae, B N F, sex Signentur Notmen Proprium

2 Mitte Bouginaria Cocainae B P C 1954, sex Signa Unus omni die utendus

3 *Recipe—*

Bismuthi Subgallatis	2 G
Resorcinolis	
Zinci Oxidi	
Balsami Peruviani	aa 1 G
Oil Theobrom q s ut fiant suppositoria sex singulum pendens fere grammata duo	

Signa Unum omni nocte adhibendum

4 *Recipe—*

Extracti Belladonnae Siccii	gr ii
Cacaonis Butyri q s ut fiat pessus pendens fere	gr ix

Mitte tales iv Signentur Unus dolore urgente utendus

5 Prepare six 1 G suppositories each containing the following—

Chloral Hydrate	200 mg
Witepsol E Base	q s

Label The Suppositories Use one at night

6 Prepare six 1 gramme suppositories each containing the following—

Ichthammol	0.3 G
Massuppol Base	q s

Label The Suppositories One to be used when required

7 Prepare six suppositories each containing 10 per cent of phenol and weighing 1 gramme (Use Massa Esterinum C Base)

Label The Suppositories Use as directed

## REFERENCES

- ALLAWALA, N A and RIEGELMAN, S (1953) The Release of Antimicrobial Agents from Solutions of Surface Active Agents *J Amer pharm Ass (Sc Ed)*, 42, 267-275
- CACCHILLO, A F and HASSSLER, W H (1945) The Influence of Suppository Bases upon the Rectal Absorption of Acetylsalicylic Acid. *ibid*, 43, 683-685
- CALDWELL, A F (1939) Hydrogenated Fats in Bases for Tropical Countries *Quart J Pharm Pharmacol*, 12, 689-698
- CHARANVAT, R., CHEVILLARD, L. and GIOVO, L (1949) Sur une nouvelle méthode d'essai physiol d'excipients de suppositoires *Ann Pharm Franc*, 7, 627-632 Abstract in *J Pharm Pharmacol*, 2, 602 The Physiological Evaluation of Suppository Excipients
- GRADNICK, M B (1955) Absorption from the Rectum *Pharm J*, 175, 316
- HASSLER, W H and SPERANDIO, G J (1953) The Formulation of a Water Soluble Suppository Base *J Amer pharm Ass (Prac Ed)*, 14, 26
- PENATTI, L and STEIGER-TRIPPI, K. (1958) Über die Beeinflussung der Resorption von rektal verabreichten Arzneistoffen durch Suppositoriengrundmassen. *Pharm Acta Helv*, 33, 663-677
- RAE, J (1942) A Suppository Base The Use of Phosphated Cetyl and Stearyl Alcohols *Pharm J*, 148, 13
- SOULSBY, J and HOPKINS, S J (1956) A Survey of Suppository Bases *ibid*, 176, 157-158
- WHITWORTH, C. W and LAROCCA, J P (1959) A Study of the Effect of Some Emulsifying Agents on Drug Release from Suppository Bases *J Amer Pharm Ass (Sc Ed)*, 48, 353-355



## Inhalations, Spray Solutions, and Throat Paints

### Containers

BOTTLES distinguishable by touch from ordinary medicine bottles are used for the above, and if they are poisons, a coloured, vertically fluted bottle is necessary. Throat paints should be placed in wide-mouthed bottles when application by means of a brush is intended. Inhalations may be dispensed in clear, oval, vertically fluted bottles.

Many of these preparations are immiscible with water, hence the containers should be used perfectly dry.

For spray solutions made with water, alcohol or similar medium, an ordinary spray is satisfactory, but when the liquid is viscid an atomiser is necessary. The liquid should always be dispensed in a separate container, to be drawn from as required.

### INHALATIONS

Some of these inhalations are simple solutions of volatile medicaments (usually volatile oils) in alcohol

or an alcoholic preparation, often Compound Benzoin Tincture, as illustrated in Exercise 12 1.

Many other inhalations for addition to hot water are aqueous preparations containing a volatile oil water, and light magnesium carbonate. The latter acts as a distributive agent, i.e. subdivides the oil sufficiently to ensure uniform dispersion of the oil upon shaking. The distributive agent does not interfere with free volatilisation of the oil when the inhalation is added to hot water, the oil must not be emulsified, as this would retard vaporisation.

If not included in the formula, light magnesium carbonate is added in the proportion of 1 grain to each 2 minims of oily substances or 2 grains of solid compound (e.g. menthol). Exercise 12 2 is typical.

### SPRAY SOLUTIONS (*Nebulae*)

These consist of solutions intended for spraying the throat and nose. They are usually simple solutions. Light liquid Paraffin and vegetable oils were formerly

#### EXERCISE 12 1

##### *Recipe—*

Oil of Pine	1 drachm
Eucalyptus Oil	1 drachm
Compound Benzoin Tincture	6 drachms

##### *Mix*

*Label* Inhale the vapour of one teaspoonful when added to one pint of hot water

#### EXERCISE 12 2

##### *Recipe—*

Menthol	5 grains
Eucalyptus Oil	60 minims
Light Magnesium Carbonate	30 grains
Water	to 1 fluid ounce

*Label* The Inhalation One teaspoonful to be added to a pint of hot water (not boiling) Inhale the vapour Shake the bottle

common as solvents but are not satisfactory since oil may enter the trachea and cause lipid pneumonia viscous solvent, e.g. glycerin, liquid paraffin, which helps to retain the medicament *in situ*

Certain of the B.P.C. glycerins are used as throat paints (listed on p. 183), and Exercise 12.5 is typical.

### THROAT PAINTS

These are usually simple solutions of substances in a

#### EXERCISE 12.3

Prepare 1 fluid ounce of Isoprenaline Spray B.P.C.

Label The Spray Solution Use as directed

The official quantities are as follows—

	Metric	Imperial
Isoprenaline Sulphate	10 G	43 <i>2</i> gr
Propylene Glycol	50 ml	1 fl oz
Sodium Metabisulphite	1 G	4 <i>2</i> gr
Purified Water	to 1000 ml	to 10 fl oz
Suitable quantities to prepare would be 30 ml or 550 m		
Isoprenaline Sulphate	0.3 G	5 gr
Propylene Glycol	1.5 ml	27 <i>1</i> m
Sodium Metabisulphite	0.03 G	4 <i>2</i> gr
Purified Water	to 30.0 ml	to 550 m

#### EXERCISE 12.4

Recipe

Ephed. Hydrochlor gr 1ss

Sod. Chlor gr ii

Chlorbutol gr i

Aquam ad 3 ss

Signa Nebula Si opus sit utenda

#### EXERCISE 12.5

Mitte Glycerini Phenolis

3 i

Signa Pigmentum Guttare ope penicilli omni secunda hora applicandum.

Follow the B.P.C. directions preparing 40 G

#### EXERCISE 12.6

Recipe—

Iodi gr vi

Potassu Iodidi gr x

Oleum Menthae Piperitae m. v

Glycerinum ad 5 i

Fiat pigmentum Signetur Cum penicillo ter in die applicandum

Method

Finely powder the Iodine and Potassium Iodide together, dissolve in a portion of the Glycerin transfer to a measure, add the Oil of Peppermint, and then rinse the mortar with sufficient Glycerin to produce 1 oz. Alternatively, about ten drops of water may be used to dissolve the potassium iodide and iodine. Solution may readily be obtained in a conical measure with a fairly wide base using a stirring rod. Transferring from a mortar is thus avoided, as the preparation may be made up to volume in the measure. The paint should be labelled 'Shake the Bottle'

Compare the following exercise

**EXERCISE 12.7**

Prepare 2 fl oz of Mandl's paint  
Label The Throat Paint Use as directed

**B P.C. GLYCERINS**

Glycerin of Tannic Acid

Tannic Acid

Glycerin

Glycerin of Borax B.P.C. 1958

Borax

Glycerin

Glycerin of Phenol

Phenol

Glycerin

All the above are prepared by warming the powdered medicament with the solvent until solution takes place, and then filtering if necessary

Glycerin of Ichthammol

Glycerin

Ichthammol

Mix

Glycerin of Starch

This is a non-greasy application for use *per se* or with a medicament, and is described on p 150

## 13

## Eye Drops and Ear Drops

## EYE DROPS

EYE DROPS are aqueous or oily solutions and ideally should possess the following properties—

They should be sterile when issued.

They should be free from foreign particles.

They should be free from irritating effect.

They should contain a suitable preservative to prevent the growth of microorganisms which may be accidentally introduced during use.

When possible aqueous solutions should be isotonic with the lachrymal secretion.

The hydrogen ion concentration should be suitable for the particular drug and, ideally, not too far from neutrality.

They should be chemically stable.

Serious damage to the eyes, even blindness, has resulted from the use of eye drops containing pathogenic organisms. Great care, therefore, must be taken in the preparation of these solutions. It should be appreciated that in use the eye drops may become contaminated. A suitable preservative will prevent the development of any microorganisms that may be introduced but the pharmacist should give advice on the use of these solutions so as to reduce contamination to a minimum.

## FORMULATION OF EYE DROPS

In addition to the medicament, eye drops may contain a number of additional substances in order to maintain potency and to prevent deterioration. These may include the following—

*Preservatives* As already stated, these are included to prevent the development of microorganisms which may be introduced during the use of the drops. A critical account of a number of these is given on pp. 188, 189. The B.P.C. specifies Solution for Eye Drops for most of the eye drops, and in others

uses phenylmercuric nitrate, phenylethylalcohol and benzalkonium chloride.

*Adjustment to Isotonicity with Lachrymal Secretion* Sodium chloride is normally used to adjust suitably the osmotic pressure of eye drop solutions. In some cases, however, the medicament renders the solution hypertonic, e.g. Eye Drops of Sulphacetamide. The eye can tolerate quite a wide variation in osmotic pressure of eye solutions but it is usual, where possible, to adjust them to isotonicity with lachrymal secretion.

*Oxidation of the Medicament* Many ophthalmic drugs are readily oxidised and it is usual in such cases to include a reducing agent. Sodium metabisulphite in a concentration of 0.1 per cent is commonly used for this purpose. It is used for example in the B.P.C. eye drops of Physostigmine, Phenylephrine and Sulphacetamide.

*Hydrogen Ion Concentration* The need for a stable hydrogen ion concentration is discussed on p. 192, and some of the buffer solutions in common use described. Once again, reference to the B.P.C. eye drops will provide examples of the use of buffering agents. Sodium citrate is used in Eye Drops of Phenylephrine, boric acid and borax in Eye Drops of Chloramphenicol and sodium acid phosphate in Eye Drops of Prednisolone Sodium Phosphate.

*Chelating Agents* When the ions of heavy metals are liable to cause decomposition of a medicament in solution a chelating agent, which binds the ions in an organic complex, will give protection. Sodium edetate is one of the best-known chelating agents and it is used in Eye Drops of Prednisolone Sodium Phosphate.

*Viscosity* To provide a viscous solution which will prolong the action of an eye solution by remaining longer in contact with the eye surface, thickening

agents may be used. Methyl cellulose 1 per cent has been used for this purpose.

### CONTAINERS

The usual type of container for eye drops is a vertically fluted amber or green glass bottle fitted with a bakelite cap carrying a dropping tube with a teat and capable of being closed so as to exclude micro organisms. The following properties are important:

- 1 They comply with the test for the limit of alkalinity of glass. Capper (1963) has shown that sometimes the bottle may be free from alkalinity but the dropper tube may not. This was demonstrated by Eye Drops of Physostigmine in which the solution in the bottle was colourless but that in the dropper tube was pink.
- 2 They protect the contents against light. Many ophthalmic drugs are light sensitive.
- 3 They have a satisfactory seal. Norton (1963) proposes a dye test.
- 4 Rubber teats and teats of other materials are absorbent and should be impregnated with any preservative used in the eye solution for which they are employed (see Chapter 21).
- 5 They provide a dropper ready for use and protected against breakage and contamination.
- 6 They comply with the poisons regulations. Many ophthalmic drugs are poisons.
- 7 Non glass containers should not react with the medicament or yield particles to the solution contained therein.

Norton (1962) describes tests which he carried out on eye drop bottles from seven sources. Only one of these sources provided bottles which were satisfactory in withstanding the steaming and autoclaving when sealed and in maintaining the seal intact throughout the processes. In further investigations Richards Fletcher and Norton (1963) carried out a more detailed investigation of eye-drop bottles. These tests showed that there is a serious need for standard specifications for eye drop bottles that silicone rubber teats are more durable to autoclaving than those of natural rubber that metal caps are more durable than bakelite caps but tend to shed fine particles along the screw thread and are incompatible with mercury compounds. In addition it was shown by a dye test that containers are available with closures which have an effective seal during and after autoclaving.

### SPECIAL DROPPING BOTTLES

Among recent attempts to devise a safe and convenient eye drop bottle is the Dropule bottle made



*Fig 131 DROPOULE BOTTLE  
(Courtesy Glaxo Laboratories)*

by Glaxo Laboratories as a container for their own particular eye drops. It is of plastic and is unbreakable. It can be readily sterilised and yields no contamination to the contained solution.

It consists of a plastic body with a plastic welded cap Fig 131 in which it will be seen that a drop is easily expelled simply by bending the cap projecting on

### THE PREPARATION OF EYE DROPS

On a large scale routine methods are used with adequate bacteriological controls. In these circumstances the production of sterile ophthalmic solutions does not present special difficulties. In the extemporaneous dispensing of these however there are the problems of limited time and often incomplete facilities for asepsis.

The BPC 1963 outlines five methods for preparing eye drops. These are—

*Method A(1)* Sterilisation by filtration followed by aseptic transfer to a sterile container which is then aseptically closed to exclude microorganisms.

*Method A(i)* Sterilisation by heating in an autoclave after filtration and sealing in the final container

*Method B* Heating at 98° to 100°C for thirty minutes after filtration and sealing in the final container. The eye drops must be freshly prepared.

*Method C* Preparation by asepsis using a sterilised aqueous vehicle, sterilised apparatus and container. The eye drops must be freshly prepared.

*Method D* Preparation by asepsis when the vehicle is an oil. This method is similar to Method C except that there is no requirement that the drops should be freshly prepared.

In hospital pharmacy departments and manufacturing laboratories all or any of these methods could be used, facilities for asepsis and time to carry out sterility tests being usually adequate. In extemporaneous dispensing, however, the preparation of half-ounce quantities or less of a particular solution presents difficulties. Some of these are indicated in the following comments on the above processes.

*Method A(i)*, when applied to the preparation of injections, must be followed by sterility tests. Where proper facilities for asepsis are available and there is time to carry out sterility tests this method is, of course, satisfactory for eye solutions.

As a recent example of this, Sutaria and Williams (1962) describe in detail the procedure they have devised for the preparation of ophthalmic solutions in which sterilisation is effected by filtration through disposable membrane filters.

For extemporaneous dispensing of small quantities

of eye drops other methods are more practical.

*Method A(ii)*, heating in an autoclave at 115° to 116°C for thirty minutes is a satisfactory method, but a number of ophthalmic drugs will not withstand this treatment and some sealed eye-drop bottles, as previously mentioned, are open to serious criticism. Experiments by one of us, however, have shown that certain bottles with their dropper assembly have proved satisfactory.\*

*Method B* may be used for most of the B.P.C. eye drops. It is a simple method requiring no specialised apparatus. Usually Solution for Eye Drops is used as the preservative vehicle. It will be appreciated that this is not as certain a method of sterilisation as heating with a bactericide which requires 0.2 per cent of chlorocresol or other equally potent bactericide. Such concentrations are not admissible in eye drops because of their irritating properties. It is, therefore, most important that the greatest possible care be taken in thoroughly cleansing apparatus and containers, and that every precaution is used in dispensing to exclude contamination.

*Method C* is stated to be suitable for eye drops containing medicaments which will not withstand heating in solution. It will be understood from reading chapter 26 that aseptic methods require considerable technical skill, and to be fully acceptable, should be followed by sterility tests. These are not intended here by the B.P.C. since eye drops made by this method are to be 'freshly prepared', i.e. issued within

\* Marketed by Hedley and Co., Leytonstone

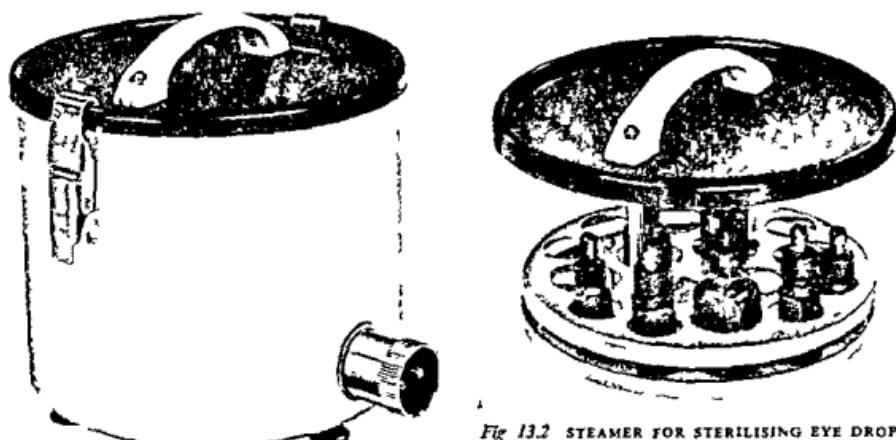


Fig. 13.2 STEAMER FOR STERILISING EYE DROPS  
(Courtesy Jacob White & Co. Ltd.)

twenty four hours of preparation. In such circumstances proper tests cannot be carried out. It is, therefore, imperative that extreme care should be taken throughout the process.

**Method D** This method also presents difficulties in extemporaneous dispensing due to the time involved, since it means heating in a hot air oven at 160°C for an hour. Time is also needed for heating the oven to 160°C, also for cooling the contents afterwards. Provision for asepsis is also necessary.

The various methods of sterilisation, involving all but Method B above, are described fully in Part 2. Method B is described below with examples.

#### Preparation of Eye Drops Using Method B

Thoroughly wash all apparatus and rinse with recently boiled and cooled Purified Water or Water for Injection. Dissolve the medicament and other ingredients in part of the vehicle and filter through a No. 3 sintered glass filter or a No. 54 Whatman filter paper to ensure a perfectly clear and particle free solution. Adjust to volume and transfer to a

dry, thoroughly cleansed, preferably sterile, eye-drop bottle, close securely and heat at 98° to 100°C for half an hour. A steamer is suitable for this purpose. A specially designed type is described below and illustrated in Fig. 13.2. Alternatively, the bottle may be placed in a water bath so that the water reaches to the neck of the bottle, and the temperature allowed to rise to boiling point. Time must then be allowed for the contents to reach the proper temperature before recording the thirty minute heating period. For the usual small eye-drop bottles, five minutes is sufficient to ensure a temperature of 98° to 100°C within the container.

#### A Steamer Designed for Eye Drop Bottles

This steamer consists of an aluminium, electrically heated water bath 6½ in in diameter and 8½ in high. It has a lid fitted with a rubber washer and, suspended from this lid on three peripheral rods, is a tray on which the eye-drop bottles are placed. This 'crucet', as it is called, is easily removed after steaming by means of a heat insulated handle.

#### EXERCISE 13.1

Prepare half a fluid ounce of Eye Drops of Atropine Sulphate B.P.C.

Label Two drops to be instilled into each eye two or three times a day  
The Codex formula is

	Metric	Imperial
Atropine Sulphate	1.0 G	4½ gr
Sodium Chloride	0.75 G	3½ gr
Solution for Eye Drops	to 100.0 ml	to 1 fl oz

Suitable quantities to prepare would be 16 ml or 275 minims (2½ × 110 min.)

Atropine Sulphate	0.16 G	2½ gr
Sodium Chloride	0.12 G	1½ gr
Solution for Eye Drops	to 16.0 ml	to 275 min

Prepare in the manner described above and dispense half a fluid ounce

#### EXERCISE 13.2

Prepare half a fluid ounce of Eye Drops of Hyoscine B.P.C.

Label One drop to be placed in the affected eye night and morning  
The Codex formula is

	Metric	Imperial
Sodium Chloride	0.85 G	3½ gr
Hyoscine Hydrobromide	0.25 G	1½ gr
Solution for Eye Drops	to 100.0 ml	to 1 fl oz

Suitable quantities to prepare would be either 20 ml or 440 minims

Sodium Chloride	170 mg	3½ gr
Hyoscine Hydrobromide	50 mg	1 gr
Solution for Eye Drops	to 20 ml	to 440 min

Prepare by the method described above and dispense half a fluid ounce

**Oily Eye-drops**

Most alkaloids as bases are soluble in castor oil, hence the latter is occasionally used as a solvent.

Solution is effected by warming and stirring the powdered alkaloid with the oil over a water-bath until dissolved (Exercise 13.3)

**EXERCISE 13.3**

Prepare 50 ml of Castor Oil containing—

Cocaine	1 in 200
Mercuric Chloride	1 in 3,000*

As mentioned above, it is necessary to warm alkaloids with castor oil in order to effect solution, a flask or dish heated over a water-bath serving best

Transfer of a measured volume of castor oil to a flask or dish would involve loss as part of the oil would adhere to the measure. Hence, greater accuracy is attained by weighing the calculated equivalent of castor oil direct into the vessel in which solution is to be effected. Therefore the Home Office issue the following instructions for making the above solution—

Weigh 95 G of Castor Oil into a flask capable of holding twice the amount. Add 0.5 G of Cocaine and warm in a water-bath until dissolved. While the solution is still warm (but not hot) add 1 ml of a solution containing 3.3 G of Mercuric Chloride in 100 ml of Dehydrated Alcohol. Mix the solutions by rotating the flask.

The specific gravity of castor oil is 0.958–0.969, therefore 95 G measure 99 ml (approx.), and the total volume of product is, therefore, 100 ml (approx.)

For 50 ml, the quantities are—

Cocaine	.	0.25 G
Castor Oil		47.50 G

Warm over a water bath and stir until dissolved

Mercuric Chloride	0.017 G
Dehydrated Alcohol	0.500 ml

Dissolve, and mix with the warm solution formed above

\* This solution is the one mentioned in the D.D.A.

**Preservatives in Eye Drops**

Preservatives for eye drops should have the following properties—

They should be capable of preventing the development of microorganisms introduced during the use of the drops.

They should be non irritant

They must be stable chemically and should be non-volatile

They must be compatible with the other ingredients of the drops

They must be well within their solubility to avoid crystallisation should they be stored at too low a temperature

**BENZALKONIUM CHLORIDE**

The U.S.P. XVI states that in a concentration of 0.01 per cent benzalkonium chloride is the agent most frequently used as a preservative in ophthalmic solutions. (This obviously refers to use in the United States of America.) In this country it is available only as a 50 per cent solution, Benzalkonium Chloride Solution B.P.C. This solution is used in B.P.C. eye drops of Carbachol, Phenylephrine and Prednisolone Sodium Phosphate in a concentration of 0.04 per cent. In the first and last of these three examples the preservative effect is enhanced by the use of 0.25 per cent of phenylethyl alcohol.

**It is non toxic, non irritant, stable in solution and non-volatile. Its surface active properties may be an aid to the absorption of the drug with which it is used.**

#### CETRIMIDE

In a concentration of 0.001 per cent cetrimide is an effective preservative.

It is soluble in two parts of water, stable in solution, non volatile and non irritant.

It is compatible with most ophthalmic drugs but not with anionic surface-active agents, iodides, phenylmercuric nitrate, phenol and chlorocresol. A concentration of 0.005 per cent has been used as a preservative in ophthalmic solutions.

#### CHLORBUTOL

Chlorbutol is preservative in concentrations of 0.5 per cent.

It is volatile and its effective concentration may, therefore, be reduced during preparation and use.

In addition, it hydrolyses in solution with the production of hydrochloric acid, a result which is increased at a raised temperature. For these reasons it is not recommended.

#### CHLOROCRESOL

In a concentration of 0.05 per cent chlorocresol is a satisfactory preservative but it is irritant and is not recommended. Even slightly irritant substances can cause intense pain when introduced into an inflamed eye. In the B.P.C. 1963 most eye drops were formulated with 0.05 per cent chlorocresol but after several adverse reports on it were received from ophthalmic practitioners it was deleted in favour of Solution for Eye Drops. This was effected by a special announcement in the *Pharmaceutical Journal* of 7th December (1963).

#### METHYLHYDROXY BENZOATE

Ridley (1958) claims that a concentration of 0.1 per cent is effective as a self sterilising fluid. It is, however, irritant to some extent.

#### PHENYLETHYL ALCOHOL

Brewer *et al* (1953) claim that phenylethyl alcohol is effective in a concentration of 0.5 per cent against *Pseudomonas pyocyanea*. Grote and Woods (1955) recommend a 0.3 per cent solution as a suitable preservative for ophthalmic solutions. They claim that it is non irritant and does not destroy the natural lysozyme present in the lachrymal secretion. It is used in a concentration of 0.25 per cent in Eye Drops.

of Carbachol and Eye Drops of Prednisolone Sodium Phosphate together with benzalkonium chloride

#### PHENYLMERCURIC NITRATE

In a concentration of 0.001 per cent, phenylmercuric nitrate is an effective preservative, non irritant, stable and non-volatile.

It is soluble in 1,500 parts of water and is, therefore, quite soluble at the required concentration.

It is incompatible with halides but at the above concentrations precipitation is unlikely even with these. No precipitation occurs with 0.9 per cent sodium chloride. Precipitation may occur with hyoscine hydrobromide and with homatropine hydrobromide. Although non-volatile, phenylmercuric nitrate is readily absorbed by rubber. For this reason attention is drawn to the requirement that dropper-bottle teats should be stored in a solution of the preservative prior to use so that no more will be taken up from the dispensed drops.

Bain (1956) has shown that a 0.0001 per cent solution is fungistatic in nutrient solution containing protein.

Ridley (1958) describes the use of 0.004 per cent solution as a self-sterilising vehicle.

Eye drops of Fluorescine contain phenylmercuric nitrate in a concentration of 0.002 per cent. This is necessary to prevent the growth of bacteria, in particular those of the genus *Pseudomonas* which are pathogenic in the eye. These organisms grow readily in fluorescine solutions.

In spite of its apparent excellence it has not been adopted generally as an eye solution preservative since it is reported that a deposition of metallic mercury on the lens capsule had occurred after using it in eye drops (*Pharmaceutical Journal*, 1963).

#### SOLUTION FOR EYE DROPS B.P.C.

This solution contains methylhydroxy benzoate, slightly over 0.02 per cent and propylhydroxy benzoate, slightly over 0.01 per cent. Although the solution is not particularly irritant it has been criticised on the grounds that it is not a satisfactory preservative. Klein, Millwood and Walther (1954) state that it is only one third of the concentration necessary to be effective against *Pseudomonas pyocyanea*. Using 93 fungal test organisms and saturated solutions of these esters Bain (1956) found that, in the methyl ester solution, 39 survived and in the propyl ester 57 survived.

For these and other reasons the solution was deleted from the B.P.C. 1963 in favour of 0.05 per cent chlorocresol. As mentioned under chlorocresol,

this was reversed later. A fully satisfactory eye solution preservative has still to be found.

### THE USE OF SELF STERILISING VEHICLES

Ridley (1958) describes a method for the preparation of eye drops which has been developed at the High Holborn Branch of Moorfields Eye Hospital. This depends on the use of chemicals to ensure that the eye drops are self sterilising. Regular routine tests are claimed to show that the method is effective in producing sterile products.

The recommended substances are Methylhydroxy Benzoate 0.1 per cent and Phenylmercuric Nitrate 0.004 per cent. It is recommended that they are used with the following medicaments:

*Methylhydroxy Benzoate* Adrenaline, amethocaine, antazoline hydrochloride, atropine methyl

nitrate, chloramphenicol, cocaine hydrochloride, cortisone, ephedrine, ethylmorphine, homatropine, hydrocortisone, hyoscine, lachesis, lignocaine, neomycin sulphate, penicillin, potassium iodide, sodium iodide, streptomycin, sulphacetamide and zinc sulphate.

*Phenylmercuric Nitrate* Atropine, copper sulphate, duobosine eserine, fluorescein, glycerin, methylcellulose, pilocarpine, sulphacetamide.

It is claimed that after three hours at room temperature such solutions are sterile even when they have been previously contaminated with such organisms as *Ps. pyocyanea*, *Streptococcus pyogenes* and *Staphylococcus aureus*.

The solutions should be made with the same strict cleanliness as any other eye solutions and filtered to ensure a perfectly particle-free product. (Exercises 13.4 and 13.5)

### EXERCISE 13.4

#### Recipe—

Homatropine hydrobromide	2.0 G
Methylhydroxy benzoate	0.1 G
Sodium chloride	0.5 G
Purified Water to	100.0 ml

Prepare 20 millilitres

Label Two or three drops in each eye as directed.

If no stock solution of methylhydroxy benzoate is available a solution containing 50 mg in 50 ml should be prepared using Purified Water. The quantities for 20 millilitres will be—

Homatropine hydrobromide	0.4 G
Sodium chloride	0.1 G
Solution of methylhydroxy benzoate 0.1 per cent to	1 ml
	20 ml

### EXERCISE 13.5

#### Recipe—

Pilocarpine nitrate	1.0 G
Sodium chloride	0.7 G
Phenylmercuric nitrate solution 0.004% to	100 ml

Prepare 20 millilitres

Label The Eye Drops One drop in the left eye occasionally.

It is convenient to prepare a quantity of 0.01 per cent solution (50 mg in 500 ml) of phenylmercuric nitrate and dilute as required for use. The quantities required for 20 millilitres will be—

Pilocarpine nitrate	0.2 G
Sodium chloride	0.14 G
Phenylmercuric nitrate solution 0.01%	8.0 ml
Purified Water to	20.0 ml

The above two solutions are prepared, filtered and bottled in the usual manner and issued after a period of three hours at room temperature.

## RAPID ADJUSTMENT TO ISOTONICITY

It will be realised that when a medicament is added to an isotonic solution the product will be hypertonic. This may not be important since the eye can tolerate a range equivalent to 0·6 to 1·4 per cent sodium chloride solution. A method widely used in the United States of America has been devised to overcome the difficulty of speedy extemporaneous dispensing. In this method the medicament is dissolved in sufficient distilled water to produce a solution isotonic with lachrymal secretion and then adjustment to volume is made with an isotonic vehicle. Methods have been devised by White and Vincent (1947), Sprouls (1949) and Greco (1952), to provide rapid means of finding the correct volume of water in which to dissolve the medicament. Such a table is included in the U.S.P. XVI.

This list gives the volume of sterile water in which to dissolve 300 mg (1 per cent of 30 ml—the usual volume prescribed) of each named medicament to produce a solution isotonic with lachrymal secretion. For strengths other than 1 per cent the volume of water is found by simple proportion.

As an example consider the following—

Atropine Sulphate	1 per cent
Sterile Water	q.s.
Normal Saline Solution to	30 ml

The figure in the U.S.P. XVI for Atropine Sulphate is 4·3 ml, therefore, 300 mg of the salt is dissolved in sterile water and adjusted to 4·3 ml. This is then diluted to 30 ml with the saline solution.

It should be noted that the volumes may be calculated from the figures in the B.P.C. Appendix 5 for the depression of freezing point of water by various medicaments. For example, if we consider the same prescription the calculation is as follows—

From the B.P.C. table it is seen that a 1 per cent solution of Atropine Sulphate freezes at  $-0\ 074^{\circ}\text{C}$ .

Since a freezing point of  $-0\ 074^{\circ}\text{C}$  is given by 300 mg in 30 ml, therefore, a freezing point of  $-0\ 52^{\circ}\text{C}$  (the freezing point of lachrymal secretion)

is given by 300 mg in

$$\frac{0\ 074}{0\ 52} \times 30 = 4\ 27\text{ ml}$$

This may be approximated to 4·3 ml.

Table 13.1, in which the figures are calculated from the freezing point figures given in the B.P.C. Appendix 5, shows the volume of water in which to dissolve 300 mg of each drug to produce a solution isotonic with lachrymal secretion. For half-ounce quantities, more usual in this country, 15 ml quantities could be made and 150 mg would therefore be dissolved in half the volumes stated.

Table 13.1

Drug	Volume of water to produce a solution isotonic with lachrymal secretion (ml)
300 mg	
Amethocaine Hydrochloride	6·2
Atropine Methonitrate	5·8
Atropine Sulphate	4·3
Carbachol	11·6
Cocaine Hydrochloride	5·2
Fluoresceine Sodium	10·4
Homatropine Hydrobromide	5·6
Hyoscine Hydrobromide	4·0
Mild Silver Protein	5·5
Physostigmine Salicylate	5·2
Pilocarpine Nitrate	7·6
Silver Protein	2·6
Streptomycin Sulphate	2·0
Sulphacetamide Sodium	7·6
Zinc Sulphate	5·0

Provided sterile water and sterile isotonic vehicles are available the solutions may be made rapidly when required. In the absence of satisfactory aseptic conditions the solution may, in most cases, be heated at  $98^{\circ}$  to  $100^{\circ}\text{C}$  for half an hour in the final sealed container.

### EXAMPLE 13.1

Homatropine Hydrobromide	2 per cent
Phosphate Buffer Solution (pH 6·8)*	$\frac{1}{2}$ fl oz

Prepare 15 ml 2 per cent of 15 ml is 300 mg. Therefore, dissolve this quantity in 5·6 ml of Purified Water and adjust to 15 ml with the buffer solution. Place half a fluid ounce in a suitable eye-dropper bottle, seal and heat to  $98^{\circ}$  to  $100^{\circ}\text{C}$  for half an hour.

\* see Table 13.2

**EXAMPLE 13.2**

Zinc Sulphate                      0.25 per cent  
Normal Saline Solution        to 1 fl oz

Prepare 30 ml 0.25 per cent of 30 ml is 75 mg. From Table 13 it is seen that 300 mg are to be dissolved in 5 ml, therefore, 75 mg will be dissolved in 1.25 ml of Purified Water. The solution will then be adjusted to 30 ml with Normal Saline Solution. One ounce will be placed in a suitable eye-dropper bottle sealed and heated to 98° to 100°C for half an hour

## BUFFERED EYE DROPS

Alkaloidal salts figure prominently among the medicaments used for the eye. They are stable in acid solution but are unstable in alkaline solution, the alkaloidal base being precipitated at a pH of about 8.3.

The hydrogen ion concentration of tears lies between 7.2 and 7.4 but alkaloidal salts are more stable at lower pH values. The physiological activity is said to be due to the alkaloidal bases rather than the salts. This means that the physiological activity will increase as the pH rises towards the point of precipitation. Irritation of the eye also increases with rise in pH.

The most satisfactory condition is one at which a balance is struck between higher activity and irritation, on the one hand, and lower activity and stability on the other. In this connexion Goyan (1947) divides the usual ophthalmic drugs into two groups, one to be preserved with a boric acid buffer at pH 5, and the other in a phosphate buffer at pH 6.8.

The United States Pharmacopoeia XVI quotes formulae for ophthalmic solutions commonly used in ophthalmic practice which are isotonic with lacrimal secretion.

The first contains 1.9 per cent of boric acid and 0.001 per cent of phenylmercuric nitrate. This is suitable for such drugs as salts of cocaine, phenylephrine, procaine, tetracaine and zinc.

The second contains, in addition, 0.1 per cent of sodium sulphite to prevent oxidation with such drugs as physostigmine and adrenaline. The hydrogen ion concentration of these solutions is just below 5.

A series of phosphate buffers is quoted by Hind and Goyan (1947) giving a range of pH values as indicated below. A similar table appears in the U.S.P.XVI

with sufficient sodium chloride to render the solutions isotonic with lacrymal secretion.

Table 13.2

	Sodium acid phosphate solution	Sodium phosphate solution	Grammes of sodium chloride per cent to render isotonic
pH	(c c)	(c c)	
5.91	90.0	10.0	0.52
6.24	80.0	20.0	0.51
6.47	70.0	30.0	0.50
6.46	60.0	40.0	0.49
6.81	50.0	50.0	0.48
6.98	40.0	60.0	0.46
7.17	30.0	70.0	0.45
7.38	20.0	80.0	0.44
7.73	10.0	90.0	0.43
8.04	5.0	95.0	0.42

It will be seen from this table that equal volumes of the two phosphate solutions give a pH of 6.8 and requires 0.48 per cent of sodium chloride for isotonicity with lacrimal secretion. This solution is suitable for salts of atropine, ephedrine, homatropine and hyoscine.

## EYE DROPS IN SINGLE-DOSE UNITS

It was inevitable that the difficulties and dangers associated with the presentation of sterile eye drops would lead to the production of single-dose containers. Cross-infection can easily occur if the usual dropping bottle is used for several patients in succession. There is, moreover, a special need for assured sterility in the use of eye drops where the surface of the eye may be damaged.

One such product is marketed by Smith and Nephew Pharmaceuticals Ltd. under the proprietary name of Minims. Originally this was produced by the Barnes-Hind Laboratories in the U.S.A.

Each unit contains a quantity for one application. The sterile drops are contained in an impermeable, pliable plastic dropper tube. The dropper is sealed

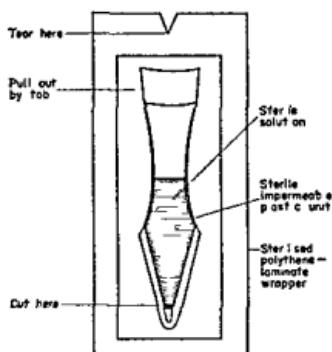


Fig. 13.3 SINGLE-DOSE UNIT EYE DROPPER

within an outer plastic envelope which maintains the sterility of the dropper and contents, even the outside of the dropper, thus reducing accidental contamination in use to a minimum.

The unit (Fig. 13.3) is used by tearing open the outer envelope, pulling out the dropper by the tab, cutting off the tip with sterile scissors, and gently squeezing the tube to eject a drop of the fluid.

Sterilisation is effected by exposure to a mixture of carbon dioxide (80%) and ethylene oxide (20%) for periods of up to 17 hours under a temperature of

60°C and a pressure of 15 lb. The length of exposure depends on the particular Minim being sterilised.

Stability of the solution is maintained by buffering, and a preservative is included which may be either *phenylmercuric nitrate* or *chlorbutol*.

Since the B.P.C. directs that eye drops should be freshly prepared these preparations do not comply, but, on the other hand, they are stable on storage.

The drops available in this form are those in common use as mydriatics, miotics, anaesthetics and for staining purposes. The list is given below. They are of obvious value in first-aid clinics, consulting rooms or operating theatres or for emergencies in general medical practice.

	per cent
Atropine Sulphate	1
Homatropine Hydrobromide	2
Hyoscine Hydrobromide	0.2
Pilocarpine Nitrate	1
Physostigmine Salicylate	0.5
Tetracaine Hydrochloride	0.5
Fluorescein Sodium	2

A fluorescein sodium unit is also available with a cotton wool tip for corneal staining.

The units are expensive if comparison is made with eye drops made in the usual way, and may not become widely used, but they are of considerable value in the special cases mentioned above.

#### Summary of B.P.C. Eye Drops

Name	Preservative	Additions	Comments
<i>Prepared by Methods A or B</i>			
Eye Drops of Atropine Sulphate	Soln for Eye Drops	Sodium chloride	
Carbachol	Phenylethyl alcohol 0.25% and Benzalkonium chloride 0.02%	Sodium chloride	Alkali free container
Cocaine	Soln for Eye Drops	Sodium chloride	
Cocaine and Homatropine	Soln. for Eye Drops	Sodium chloride	
Fluorescein	Phenylmercuric nitrate 0.002%	Sodium chloride	
Homatropine	Soln for Eye Drops	Sodium chloride	
Hyoscine	Soln for Eye Drops	Sodium chloride	Alkali free container
Lachesine	Soln for Eye Drops	Sodium chloride	
Pilocarpine	Soln. for Eye Drops	Sodium chloride	
Zinc Sulphate	Soln for Eye Drops	Sodium chloride	
Zinc Sulphate and Adrenaline	Chlorbutol	50% Adrenaline Solution providing Chlorocresol 0.05% Chlorbutol 0.02% Sodium metabisulphite 0.05% Sodium chloride 0.4%	Protect from light Store in cool place
<i>Prepared by Method B only</i>			
Eye Drops of Amethocaine	Soln for Eye Drops	Sodium chloride	Protect from light <i>(continued overleaf)</i>

*Summary of B.P.C Eye Drops continued*

Name	Preservative	Additions	Comments
<i>Prepared by Methods A(i) or C</i>			
Eye Drops of Atropine Methonitrate	Soln. for Eye Drops	Sodium chloride	Freshly prepared Protect from light
Mild Silver Protein	Soln. for Eye Drops		Freshly prepared Protect from light
Phenylephrine	Benzalkonium chloride 0.02%	Sodium metabisulphite Sodium citrate	Freshly prepared Protect from light
Physostigmine	Soln. for Eye Drops	Sodium chloride	Protect from light
Silver Protein	Soln. for Eye Drops	Sodium metabisulphite	Freshly prepared Protect from light
Sulphacetamide	Soln. for Eye Drops	Sodium metab sulphite	Freshly prepared Protect from light
Sulphacetamide Strong	Soln. for Eye Drops	Sodium metab sulphite	Protect from light
<i>Prepared by Method D</i>			
Eye Drops of Physostigmine Oily	None		Vehicle—Castor Oil
<i>Prepared by other methods</i>			
Eye Drops of Chloramphenicol	Soln. for Eye Drops	Boric acid Borax	Chloramphenicol dissolved in a solution of other ingredients prepared by Method B and cooled to 60°C. Volume adjusted aseptically Freshly prepared.
Prednisolone Sodium Phosphate	Phenylethyl alcohol 0.25% and Benzalkonium chloride 0.02%*	Sodium chloride Sodium acid phosphate Sodium edetate Sodium hydroxide	Method of preparation is basically Method B after adjusting to pH 8

**EAR DROPS (AURISTILLAE)**

These are solutions to be instilled into the ear, and may be solutions of drugs in water, diluted alcohol, glycerin or propylene glycol. When water is the solvent, Purified Water, boiled and cooled should be used. Containers and apparatus should al

ways be thoroughly cleansed and rinsed before use. Eye-dropper bottles may be used for ear drops.

There are no special difficulties in the preparation of ear drops. They should be labelled, in addition to any special direction, "For External Use Only".

EXERCISE 13 6	Chloramphenicol Propylene glycol	5% q.s
Prepare and send $\frac{1}{2}$ fluid ounce		
Label The Ear Drops Three drops to be placed in the affected ear as directed.		
It will be convenient to prepare 15 ml, requiring 750 mg of chloramphenicol or 275 minims ( $2\frac{1}{2} \times 110$ m) requiring 12 $\frac{1}{2}$ grains		

EXERCISE 13 7	Boric Acid Alcohol 95% Purified Water to	2.0% 20.0% 100.0%
Prepare 15 millilitres		
Label Three or four drops to be placed in each ear occasionally. Dissolve the boric acid in about 11 ml of Purified Water, add the alcohol and adjust to volume with Purified Water		

**EXERCISE 13.8**

Prepare 1 fluid ounce of Phenol Ear Drops B P C

Label To be used for the affected ear as directed

An additional label, 'Not to be diluted with water' may be added since dilution with water makes the solution caustic

**REFERENCES**

- BAIN, E. G (1956) *M Sc Thesis, Manchester* An Investigation of the Characters, Development and Inhibition of Fungal Contaminants in Aqueous Ophthalmic Solutions
- BREWER, J. H., GOLDSTEIN, S. W. and MC LAUGHLIN, C. B (1953) Phenylethyl Alcohol as a Bacteriostatic Agent in Ophthalmic Solutions *J Amer pharm Ass (Sci Ed)*, 42, 584-585
- CAPPER, K. R (1963) Containers for Medicines *Pharm J*, 191, 520
- EDISMORE, C. T (1957) Ophthalmic Solutions A review article *Guildcraft*, 31, 21-32
- GRECO, S. J (1952) Isotonic Solutions A Simplified Method of Preparation *J Amer pharm Ass (Prac Ed)*, 13, 340
- GROTE, J. W. and WOODS, M (1955) Beta-phenylethyl Alcohol as a Preservative for Ophthalmic Solutions *J Amer pharm Ass (Sci Ed)*, 44, 9
- HADGRAFT, J. W (1962) A Single Dose Disposable Container for Eye Drops *Ophthal Opt*, 2, 75
- HIND, H. W. and GOYAN, F. M (1947) A New Concept of the Role of Hydrogen Ion Concentration and Buffer Systems in the Preparation of Ophthalmic Solutions *J Amer pharm Ass (Sci Ed)*, 36, 33
- HIND, H. W., GOYAN, F. M and SCHWARTZ, T. M (1947) Notes on the Role of Hydrogen Ion Concentration and Buffer Systems in the Preparation of Ophthalmic Solutions *Ibid*, 36, 413
- KLEIN, M., MILLWOOD, E. G and WALTHER, W. W. (1954) On the Maintenance of Sterility in Eye Drops *J Pharm Pharmacol*, 6, 725
- NORTON, D. A (1962) The Properties of Eye Drop Bottles *Pharm J*, 189, 86-87
- RICHARDS, R. M. E., FLETCHER, G and NORTON, D. A (1963) Closures for Eye Drop Containers *Pharm J*, 191, 605-607
- RIDLEY, F (1958) Sterile Drops and Lotions in Ophthalmic Practice *Brit J Ophthal*, 42, 641-654
- SPROWLS, J. B (1949) A Further Simplification in the Use of Isotonic Diluting Solutions *J Amer pharm Ass (Prac Ed)*, 10, 348-350
- SUTARIA, R. H. and WILLIAMS, F. H (1962) A New Approach to the Preparation of Ophthalmic Solutions *Pharm J*, 188, 91-92
- WHITE, A. I. and VINCENT, H. C (1947) Diluting Solutions, in Preparation of Adjusting Solutions *J Amer pharm Ass (Prac Ed)*, 8, 406-411

## 14

## Gelatin Capsules

THE use of hard gelatin capsules for concealing the taste of solids has already been mentioned under Powders. Hard capsules are also very convenient for liquids, but it is more usual to dispense liquids in soft elastic capsules, which are made in a range of sizes from 5 to 30 minims. They are suitable for oils and oily bodies, but not for aqueous liquids. Creosote and guaiacol are miscible with glycerin, and ultimately soften the envelope of the capsule, which then collapses. These liquids should, therefore, be mixed with 1½–2 volumes of a fixed oil, e.g. arachis or olive oil. Liquid extracts, other than Extract of Male Fern, must be evaporated to a semi-fluid consistency to remove water and alcohol, and should then be diluted, usually to 10 minims, with olive oil or liquid paraffin, to form a homogeneous mixture.

**METHOD****STAGE 1**

Fit up a 10 or 20 c.c. syringe with a wide bore (intraspinal) needle, and select the capsules to be filled. They are regarded as holding the stated quantity when filled to the bottom of the neck, and it is therefore unnecessary to measure the liquid. The necks may be rather long and in that case they should be trimmed with scissors to 3/16th in before filling.

**STAGE 2**

Draw the liquid into the syringe and fill each capsule to the bottom of the neck. The liquid should not be ejected until the needle is almost touching the base of the capsule. Withdraw the needle, taking care not to soil the inside of the neck, as this hinders effective sealing.

**STAGE 3**

Stand the filled capsule firmly in an upright position, e.g. in a suppository mould, and thoroughly wipe

the needle to prevent the liquid soiling the lip of the next capsule. Repeat until all the capsules are filled.

**STAGE 4**

Prepare a small quantity of 20 per cent aqueous solution of gelatin and allow to cool until it becomes syrupy. Heat a metal spatula in a Bunsen flame for a few moments and apply it to the open neck of the capsule in order to soften it. Using a thin glass rod add a drop of the gelatin mass to the neck so that it forms a neat convex seal. The drop mixes readily with the softened gelatin of the neck and on cooling becomes a strong seal. Practice is necessary to determine the right temperature for ensuring a smooth finish.

Allow the capsules to cool for about 10 minutes, and then test the sealing by gentle pressure.

Pack between waxed paper in a pill or powder box.

An alternative method of sealing (suggested by S J Starkey, *PJ* Feb 27, 1937, page 218) is as follows—

Fit a boiling tube with a 1-hole cork through which passes a glass tube bent at right angles and with the outer end drawn out to a fine jet. Place water in the boiling tube and heat over a small Bunsen flame. Hold the capsule to be sealed so that its neck is in the dry part of the issuing jet of steam and slowly rotate the capsule until sealed. By this method a neat strong seal is obtained without risk of charring.

As mentioned above, hard gelatin capsules may be used in place of soft for enclosing liquids. The method is as follows—

Draw into a syringe the exact quantity of liquid to be placed in each capsule. Select a size of capsule the body of which is likely to be just filled by the measured volume, and inject the volume into the capsule. If just filled, or nearly filled, the remaining

**EXERCISE 14.1***Recipe—*

Creosot*i*  
Fiat capsula Mitte xii Signetur Una bis in die ex aqua deglutienda  
Make up to 5 minims with almond oil and measure out for 15, as follows—

Creosote	m ij
Almond Oil	30 minims
Proceed as above	to 75 minims

**EXERCISE 14.2***Recipe—*

Extracti Filicis  
Fiat capsula Mitte tales quatuor Signetur Dentur due hac nocte  
et reliquum cras nocte si opus sit  
Use hard gelatin capsules for this exercise, and proceed as above

capsules should be filled to the same level without further measurement Finally, moisten the edge of the body with water, using a camel hair brush, and press on the cap

**LARGE SCALE MANUFACTURE OF SOFT CAPSULES****Rotary Capsule Machine**

This modern machine has two, side-by side cylinders in each of which half moulds are cut. These cylinders like the rollers of a mangle, rotate in contrary directions and as they are mirror images the moulds come together precisely during rotation Two ribbons of gelatin are fed between the rollers and, just before the opposing moulds meet, jets of medicament press the gelatin ribbon into the moulds filling each half The moment of pressure follows immediately sealing the two halves together to form a capsule

These rotary machines are capable of producing between 25,000 and 30,000 capsules an hour with an

accuracy of dosage of approximately  $\pm 1$  per cent The official tolerance is  $\pm 5$  per cent.

**Seamless Gelatine Capsules**

Another modern method for making soft capsules takes advantage of the phenomenon of drop formation The essential part of the apparatus consists of two concentric tubes Through the inner tube flows the medicament and, through the surrounding outer tube, the gelatin solution The medicament, therefore, issues from the tube surrounded by gelatin and forming a spherical drop This is ensured by allowing the drop to form in liquid paraffin in which the gelatin is insoluble Regular induced pulsations cause drops of the correct size to be formed and a temperature of  $4^{\circ}\text{C}$  ensures that the gelatin shell is rapidly congealed The capsules are subsequently degreased and dried

Sizes vary from 10 mg to over 550 mg and average sizes capsules of 150 mg can be produced at a rate of 29,000 an hour Since these are not sealed by pressure from two sheets of gelatin they are seamless An accuracy of  $\pm 1.5$  per cent is claimed for this method

## 15

## Pastilles and Lozenges

## PASTILLES

PASTILLES consist of a base of glycogelatin containing a medicament in solution or suspension. As the medicament is intended to have a prolonged local action the bases should be firm enough to ensure that the pastilles dissolve very slowly.

## Base

Glycogelatin, the B.P.C. 1949 pastille base, is fairly satisfactory provided the larger proportion of gelatin suggested in that work is used, but an improved base has been made to the following formula\*—

Gelatin	1
Glycerin, by weight	2½
Water	2½

## Moulds

There are two types of moulds used in making pastilles

metal moulds and starch moulds

## METAL MOULD

These consist of a number of saucer-shaped pieces of metal fixed on a plate. The cavities, after slight

\* For large-scale manufacture a base containing acacia is used to ensure hardening.

lubrication with liquid paraffin, are filled with melted base to the brim, to ensure uniformity in size.

## STARCH MOULD

These are used in large-scale preparation. Trays measuring about 24 in. × 36 in. × 2 in. are fitted with lids to which are attached several rows of vulcanised rubber pastille shapes. The trays are filled with dried starch, the surface levelled, and the lid pressed down and then carefully raised. Depressions are thus formed in the starch corresponding to the rubber shapes. The melted pastille mass is then filled into these depressions by special plant, and allowed to harden. The adherent starch is then removed by rapid washing, and the pastilles dried.

The starch mould is readily improvised for extemporaneous work by cutting a pastille shape from hard paraffin and mounting it on a dissecting needle.

It has, however, the disadvantage that starch clings rather tenaciously to the pastilles, and time is not always available for the drying process.

For either method the capacity of the mould must be determined by filling three or four with the melted base, allowing them to set, and then finding the mean weight.

## EXERCISE 15.1

Prepare 4 oz of Throat Pastille Mass to the following formula—

Gelatin	1
Glycerin, by weight	2½
Water	2½

Cover the gelatin with the water, set aside for about 10 minutes to soften, add the glycerin and heat on a water-bath, stirring occasionally, until the gelatin dissolves, do not adjust to the original weight with water, as it is preferable to produce a base as firm as possible.

The quantity of medicament in pastilles is usually small, and, as some degree of approximation is permissible, there is no need to determine the displacement value of the medicament. Small quantities of alkaloidal salts should be dissolved in a small quantity of water before adding to the base, thereby ensuring proper distribution.

#### Packing

Pastilles are sent out in flat boxes with hinged lids, preferably made of metal. The box should be lined

with waxed paper, and the pastilles placed in layers, each layer being separated by a piece of waxed paper.

#### Uniformity of Weight

The B.P.C. directs that this is determined as follows—

Find the average weight by weighing 20 pastilles. No pastille should deviate from the average weight by more than 15 per cent and not more than one of them by more than 10 per cent.

#### EXERCISE 15 2

Send 24 pastilles each containing—

Menthol	$\frac{1}{16}$ grain
Oil of Eucalyptus	$\frac{1}{2}$ minim

Prepare in a metal mould. It is necessary to weigh out for a few more pastilles than required, therefore prepare for 30. Assuming the mould holds 25 grains of pastille mass, which must be determined beforehand, the formula will be—

Menthol	$1\frac{1}{2}$ grains
Oil of Eucalyptus	15 minims

Dissolve, and add to  $30 \times 25 = 750$  grains of melted base.

#### EXERCISE 15 3

Recipe—

Ammonii Chloridi	gr 1
Extracti Glycyrrizae Liquid.	m 1

Glycogelatini, quantum sufficiat ut fiat pastillus

Mitte viginti quatuor Signentur Unus sexies in die sugendus

Prepare in a starch mould as directed above. Weigh out for 30, dissolve the Ammonium Chloride in the melted base, and add the Liquid Extract of Liquorice.

## LOZENGES

Lozenges or troches consist chiefly of sugar and gum, medicated with a substance usually having a local action on the throat. In a few cases, e.g. Compound Lozenge of Bismuth, they afford a convenient method of administering the drug. The following description is of the traditional method of preparation. Lozenges are usually made by compression today. See under 'Tablets'.

#### Apparatus

The apparatus used is called a lozenge board, and several types are available, one being shown in Fig. 15 1. In this, two slightly tapered bars fit into the sloping groove on each side of a thick board, above which they project to a height which

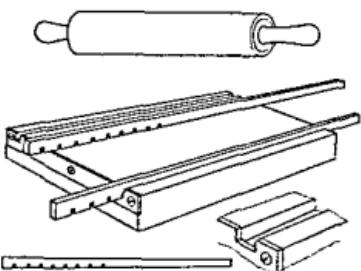


Fig. 15 1

is slightly altered by moving them along the grooves. On the underside of the bars are numerous saw cuts which fit over plates fixed across the ends of the grooves thereby retaining the bars in the desired position.

The roller rides on the projecting edge of the bars and rolls out the lozenge mass to uniform thickness, which can be finely adjusted as mentioned above.

The lozenges are cut from the rolled cake by means of punches. An improved form of punch is the Franciscus Lozenge cutter, which, by means of a spring plunger, ejects the lozenge after cutting.

#### METHOD

The most important stage in the preparation of lozenges is the formation of the lozenge mass. Powdered Acacia and water are included in all B.P.C. lozenges because they unite the ingredients to form a mass sufficiently plastic to be rolled and cut without crumbling. The quantity of ingredients should be enough to produce about 15 per cent more lozenges than required, e.g. prepare sufficient mass for 100 lozenges to fill an order for 80-90.

#### STAGE 1

Knead the components in a mortar to produce a mass of the required consistency and record its weight say  $M$  grammes for 100 lozenges.

#### STAGE 2

Roll out the mass\* to uniform thickness, cut a trial lozenge, and record its weight, which, in the present example, should be  $\frac{M}{100}$  gramme. If above or below, the metal bars must be adjusted accordingly and the mass re-rolled until the weight of a trial lozenge is exactly  $\frac{M}{100}$  gramme.

#### STAGE 3

Cut out from the cake as many lozenges as possible, re-mass and re-roll the skeleton framework to provide a further batch.

#### STAGE 4

Place the lozenges on a slab dusted over with starch and dry for 24 hours in a hot air oven at 40°C to obtain uniform hardness. Pack similarly to pastilles.

#### Uniformity of Weight

The B.P.C. directs that this is determined as follows—

Find the average weight of 20 lozenges. When weighed singly no lozenge should deviate from the average weight by more than  $\pm 15$  per cent and not more than two by more than  $\pm 10$  per cent.

#### EXERCISE 15 4

Send 1 oz of Compound Bismuth Lozenges B.P.C.

Label One to be sucked four times a day

Prepare one-twentieth of the pharmacopoeial quantity

#### EXERCISE 15 5

Mitte Trochuscos Glycyrrhiza B.P.C. quadraginta

Signentur Unus omni hora lente sugendus

Prepare sufficient mass for 50 lozenges

#### Lozenges of the B.P.C.

##### Lozenges Made by the Traditional Method

Two formulae for lozenges made by this method are included in the B.P.C. In each case the basis used contains sucrose and acacia. This is Simple Basis.

##### COMPOUND BISMUTH LOZENGES

Calcium Carbonate

Bismuth Carbonate

Heavy Magnesium Carbonate

Rose Oil of commerce

Simple Basis

##### LIQUORICE LOZENGES

Synonym Brompton Cough Lozenges

Liquorice Extract

Anise Oil

Simple Basis

\* The lozenge board may be dusted with powdered talc to prevent the mass sticking.

**Lozenges Made by Compression****BENZALKONIUM LOZENGES**

Menthol

Thymol

Eucalyptus Oil

Lemon Oil

Benzalkonium Chloride Solution

Prepared by Moist Granulation and compression

**COMPOUND BENZOCAINE LOZENGES**

Benzocaine

Borax

Menthol

Granules of benzocaine and borax are made by Moist Granulation. The menthol is dissolved in a little alcohol (95 per cent) and added to the dried granules.

**FORMALDEHYDE LOZENGES**

Synonyms Formalin Throat Tablets, Formamunt Tablets

Citric Acid

Paraformaldehyde

Menthol

Lemon Oil

Granules of citric acid with, for example, sucrose and acacia are made by Moist Granulation. The paraformaldehyde is mixed with the dried granules followed by the menthol and lemon oil dissolved in a little alcohol (95 per cent).

**HYDROCORTISONE LOZENGES**

These are prepared by Moist Granulation and compression. If no strength is specified lozenges containing 2.5 mg of hydrocortisone sodium succinate are supplied.

**PENICILLIN LOZENGES**

Synonym Benzylpenicillin Lozenges

Granules of sucrose, lactose or a mixture of the two are prepared with suitable binding agents by Moist Granulation. Benzylpenicillin is added to the dried granules and intimately mixed and the mixture compressed. Lozenges containing 1000 units are supplied if no strength is stated.

## Incompatibility

**INCOMPATIBILITY** may be physical, chemical, or therapeutic. The latter is the responsibility of the physician and includes the prescribing of substances which are of opposing pharmacological action.

In general, incompatibility is the result of prescribing together substances which are antagonistic and affect the *safety, purpose or appearance* of a product. Such effects may be physical or chemical.

### Physical Incompatibility

This is usually due to immiscibility, and many examples have already been noted, together with methods for overcoming the phenomenon. For example—

1 Oils are immiscible with water, this form of incompatibility being overcome by emulsification.

2 Resins are insoluble in water, and are therefore precipitated when added thereto in solution, e.g. in the form of resinous tinctures. If no additional substance is added the particles of resin may agglomerate and form indissoluble clots, and therefore a suitable thickening agent is usually added to correct this form of incompatibility.

There are many other instances where precipitation follows alteration in solvent, for example chlorophyll is precipitated when certain 'leaf' tinctures, notably *Hyoscyamus Tincture*, are added to water, also glyceryl trinitrate (nitroglycerin) is precipitated when Solution of Glyceryl Trinitrate is added to aqueous liquids.

3 Certain powders are immiscible with water alone, e.g. salol, phenacetin or precipitated sulphur. With these, immiscibility is countered by the addition of a suitable proportion of a thickening agent or surface active agent.

4 Certain solids, when mixed, yield oily liquids, e.g. any two of the following. Camphor, Naphthal, Phenol, Chloral Hydrate, Menthol, Thymol, also Phenazone and Sodium Salicylate.

### Chemical Incompatibility

It occasionally happens that chemical changes occur upon combining the prescribed substances, with the formation of a harmful or even dangerous product.

In such cases the prescriber must be notified whenever possible, without, of course, allowing the patient to suspect anything untoward, when the prescriber cannot be referred to, the pharmacist himself must decide the most suitable line of action.

Leaving out of consideration the above cases, which are comparatively rare, most other instances of unintentional incompatibility fall into two groups—

(a) *Tolerated*, i.e. interaction is minimised where practicable, but no alteration is made in the composition of the preparation, or

(b) *Adjusted*, i.e. interaction is prevented by some addition or substitution which does not affect the medicinal action of the preparation.

The special methods used for adjustment are well established, and it is unnecessary to refer to the prescriber. These methods will be described when they arise in the present section.

The cases where incompatibility is tolerated are frequently those in which two soluble components react to form an insoluble compound which is precipitated, and for these the following methods are often applicable.

### GENERAL METHODS FOR PRECIPITATE-YIELDING COMBINATIONS

In general, reaction between *dilute* solutions leads to precipitation in a lighter and more diffusible form than with *strong* solutions, hence the reacting substances should be diluted to the maximum extent before admixture. The resultant precipitate may be

diffusible or indiffusible, in the latter case a thickening agent should be included with one of the reacting substances. For convenience of reference the methods adopted in these two cases will be discussed as Method A and Method B.

#### METHOD A

Divide the vehicle into two equal portions, dissolve one of the reacting substances in each portion, and then mix.

This method is applied not only for diffusible precipitates but also in those cases where the amount of precipitate is very small, and where immediate re-solution of a precipitate is desired.

#### METHOD B

Divide the vehicle into two equal portions and dissolve one of the reacting substances in one

portion. To the other add, by trituration in a mortar, Compound Tragacanth Powder (10 g per oz, or 2 G per 100 ml of finished product) and then the other reacting substance, unite the two portions.

This method is applied when the precipitate is indiffusible, and forms an appreciable proportion of the mixture.

Whether Method A or Method B has been adopted, it is equally imperative to attach a 'Shake the Bottle' label, and to be sure that the patient realises the importance of this direction. To illustrate, some of the mixtures dispensed by Method A may contain a small but toxic quantity of a barely perceptible precipitate (e.g. strychnine, strychnine hydroiodide) and, unless the mixture is shaken on each occasion, it may retain nearly all the precipitate until the final doses—with possibly serious results.

### Alkaloidal Incompatibility

#### 1 ALKALOIDAL SALTS WITH ALKALINE SUBSTANCES

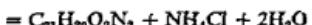
Most alkaloidal salts are soluble in water, but the alkaloids *per se* (i.e. bases) are practically insoluble.

Alkaloidal salts are decomposed by alkaline substances with formation of the alkaloid. For example, if strychnine hydrochloride is dissolved in water and solution of ammonia added, the following reaction takes place—



Strychnine Hydrochloride

407



Strychnine

334

The principal alkaline substances used in medicine are—

Aromatic Spirit of Ammonia B P C	Calcium Hydroxide Solution
Solution of Ammonia	Potassium Bicarbonate
Ammonium Bicarbonate	Potassium Hydroxide Solution
Borax	Sodium Bicarbonate

#### Solubility of Alkaloids in Water

It has been mentioned above that alkaloids are practically insoluble in water, but they are all slightly soluble, hence they are not always precipitated as will be shown from some common examples—

#### STRYCHNINE

Strychnine Hydrochloride Solution contains 1 grain of strychnine hydrochloride in 110 minims. From the above equation it is evident that—

110 minims of the official solution will yield  $\frac{1}{110}$  grains of strychnine.

The solubility of strychnine in water is about 1 in 7,000.

1 grain of strychnine dissolves in 7,000 grains of water

$$= \frac{7\ 000}{437\ 5} \text{ oz of water}$$

$\frac{334}{407}$  grain of strychnine dissolves in

$$= \frac{7\ 000 \times 334}{437\ 5 \times 407} \text{ oz of water}$$

$$= 13\ 13 \text{ oz of water}$$

i.e. the strychnine yielded by 110 minims of Strychnine Hydrochloride Solution dissolves in 13 13 ounces of water.

1 oz of water dissolves the strychnine yielded by  $\frac{110}{13\ 13} = 8\ 3$  minims of the B P C solution.

Hence strychnine is precipitated only when more than 8 minims of Strychnine Hydrochloride Solution per ounce is combined with an alkaline substance.

Strychnine is much more soluble in alcohol than in water, the solubility being 1 in 150 and it follows therefore that, in a mixture containing an appreciable proportion of alcohol, strychnine will not be precipitated even when much more than 8 minims per

ounce of Strychnine Hydrochloride Solution is combined with an alkaline substance

Nux Vomica Tincture contains only 0.125 per cent of strychnine, i.e. approximately one sixth of the strychnine present in Strychnine Hydrochloride solution, hence if the tincture were aqueous it would be necessary to have at least 48 minims per ounce before precipitation could take place if an alkaline substance were present. The tincture is, of course, alcoholic, and if 48 minims per ounce were present the alcohol contained therein would form an appreciable proportion of the preparation. It may therefore be stated that there is no likely combination of Nux Vomica Tincture and an alkaline substance from which precipitation of strychnine would ensue.

#### MORPHINE

This alkaloid is slightly soluble in water, and by calculation on similar lines it can be shown that morphine is not precipitated when 12 minims or less of Morphine Hydrochloride Solution per ounce is combined with alkaline substances.

Opium Tincture contains about one-third more morphine than the official solutions, hence morphine is not precipitated when 9 minims or less per ounce is present with an alkaline compound.

Morphine also is soluble in alcohol (1 in 100), and in a mixture containing an appreciable proportion of alcohol it will not therefore be precipitated even when much more than 12 minims per ounce of the official solutions (or 9 minims per ounce of Opium Tincture) is prescribed with an alkaline substance.

Further, morphine is soluble in solutions of hydroxides (other than ammonium hydroxide), hence precipitation is impossible with these.

#### CODEINE

This alkaloid dissolves 1 in 80 in water and is very soluble in alcohol.

The maximum dose of Codeine Phosphate, the salt mostly used, is 1 grain, and this yields about  $\frac{1}{2}$  grain of codeine itself, which will dissolve in about 1 drachm of water. The volume of mixtures for adults is usually  $\frac{1}{2}$  oz., therefore a combination in which codeine will be precipitated is unlikely.

#### CAFFEINE

This alkaloid is fairly soluble in water—about 1 in 80 or  $5\frac{1}{2}$  grains per ounce. Caffeine Citrate is the salt mostly used, and it contains 50 per cent of caffeine. The maximum dose of caffeine citrate is 10 grains, and upon decomposition this would yield 5 grains of caffeine. Hence there would be no precipitation of

caffeine with as much as 10 grains of caffeine citrate per ounce with an alkaline substance. Caffeine and Sodium Iodide contains 47–50 per cent of caffeine, hence approximately the same applies thereto.

Caffeine, also, is more soluble in alcohol (1 in 40) than in water.

#### QUININE

This is only slightly soluble in water. The salts are administered in doses upwards of 600 mg, hence in almost all cases quinine is precipitated when a normal dose of a soluble quinine salt is prescribed with an alkaline substance.

Quinine is very soluble in alcohol, and in solution of ammonia—cf. Ammoniated Quinine Solution B.P.C. It is, however, unlikely that a prescribed combination of a quinine salt with alcohol and ammonia would contain sufficient of these to effect solution of the quinine.

#### ATROPINE AND HYOSCYAMINE

These are soluble about 1 in 500 in water, and very soluble in alcohol. Belladonna Tincture contains only 0.03 per cent of Belladonna alkaloids (hyoscyamine with some atropine) and its maximum dose (30 minims) therefore contains only about  $\frac{1}{10}$  grain of alkaloid, and this would dissolve in less than 5 minims of water. Hence, precipitation of alkaloid from Belladonna Tincture, Belladonna Liquid Extract, and also Hyoscyamus Liquid Extract or Tincture is impossible in any concentration likely to be encountered in a prescription.

#### COCAINE

This is slightly soluble in water (1 in 1,300) and fairly soluble in alcohol (1 in 10). The hydrochloride is the salt principally used, ranging from 1 to 10 per cent solutions. Hence the addition of an alkaline substance to these solutions will bring about precipitation of cocaine.

Borax, which is included in the list of alkaline substances on p. 203, is sometimes prescribed with cocaine hydrochloride for use as a spray. Borax alone would bring about precipitation of cocaine which, however, may be avoided by adding half as much boric acid as borax prescribed, these two being dissolved together before the cocaine hydrochloride is added. The reaction of borax is altered in the presence of glycerin, and when this is present, borax is compatible with cocaine hydrochloride provided the latter is added last.

#### Summary

The conditions under which precipitation of certain common alkaloidal salts and preparations thereof

may occur when combined with alkaline substances may be summarised as shown in Table 16.1

Exercises 16.1, 16.2 and 16.3 are typical 'tolerated'

combinations of alkaloidal compounds with alkaline substances, and they also illustrate the three methods of treatment

Table 16.1

<i>Alkaloid or alkaloidal salt</i>	<i>Limit per oz of mixture for nonprecipitation with alkaline substances</i>	<i>De-limiting factors</i>	<i>Method (page 202) adopted when precipitation is likely</i>
Strychnine Hydrochloride Solution	8 minims	Alcohol increases solubility of Strychnine	A
Nux Vomica Tincture	Unlimited	—	—
Morphine Hydrochloride Solution	12 minims	Alcohol increases solubility of morphine—hydroxides (other than ammonium) dissolve it	A
Codeme	5 grains	Alcohol increases solubility	
Caffeine Citrate	11 grains	As for Morphine above	B
Caffeine and Sodium Iodide			
Soluble Quinine Compounds	Very small, hence with normal doses precipitation always occurs		B
Belladonna Tincture	Unlimited	—	
Belladonna Liquid Extract			—
Hyoscyamus Tincture			—
Hyoscyamus Liquid Extract			
Cocaine Hydrochloride	Very small, hence in normal concentrations precipitation always occurs	Borax with glycerin, or with Boric Acid is compatible	Refer back to prescriber

## EXERCISE 16.1

## Recipe—

Liquoris Strychniae Hydrochloridi  
 Ferri et Ammonii Citratis  
 Spiritus Ammoniae Aromatici  
 E quam ad  
 Fiat mustra Signa Cochleare magnum post singulos cibos sumendum

m. xxxvi

3 i

3 ii

3 vi

This mixture contains only 6 minims per ounce of Strychnine Hydrochloride solution, further, some alcohol is present, therefore although strychnine is formed it will not be precipitated. Consequently the *Method for Simple Mixtures*, p. 40, is satisfactory for this prescription, and a 'Shake the Bottle' label is unnecessary

**EXERCISE 16 2***Recipe—*

Liquoris Strychnine Hydrochloridi	3 iss
Spiritus Ammoniae Aromatici	3 i
Aquam	ad 3 iv

Fiat mixtura Signetur Cochlearie medium bis in die capiendum.

This mixture contains 22½ minims per ounce of Strychnine Hydrochloride Solution, and the proportion of alcohol is negligible consequently strychnine will be formed, and precipitated. Therefore follow *Method A for Precipitate-yielding Combinations*, p 203

**EXERCISE 16 3***Recipe—*

Ferri et Quininae Citratis B P C 1954	4 G
Spiritus Ammoniae Aromatici	8 ml
Aquam	ad 90 ml

Misce fiat mixtura Signa 8 ml nocte manequ sumenda.

Most of the quinine formed in this mixture will be precipitated, and will soon become indissoluble. Therefore follow *Method B for Precipitate-yielding Combinations* p 203

**2 ALKALOIDAL SALTS WITH TANNIC ACID**

Most alkaloids form insoluble tannates—a fact which is frequently utilised in the treatment of poisoning with an alkaloid, the latter being converted to its tannate. Hence, administration of a solution of tannic acid or strong tea, which contains tannin renders the alkaloid insoluble and less harmful.

Alkaloidal tannates are usually diffusible hence if practicable *Method A for Precipitate-yielding Combinations* is followed.

Tannin is fairly widely distributed, the following preparations containing sufficient tannin to react with alkaloids—

Tincture of Catechu      Hamamelis Liquid  
B P C.                      Extract

also preparations of Cinchona, Cinnamon and Wild Cherry

**3 ALKALOID SALTS WITH SOLUBLE IODIDES AND BROMIDES**

Some alkaloids, notably strychnine morphine, and codeine, form insoluble hydrobromides and strychnine forms a very insoluble hydriodide. Hence when a soluble bromide or iodide is combined thus, *Method A for Precipitate-yielding Combinations* must be followed.

**4 ALKALOIDAL SALTS WITH SALICYLATES AND BENZOATES**

A few alkaloids, notably quinine, form insoluble salicylates and benzoates.

Quinine compounds are frequently prescribed with salicylates, and as precipitated quinine salicylate is indissoluble, *Method B for Precipitate-yielding Combinations* should be followed.

**EXERCISE 16 4***Recipe—*

Quininae Hydrochloridi	gr ii
Sodi Salicylatus	3 i
Aquam	ad 3 iii

Fiat mixtura Signa Cochlearie magnum inter cibos sumendum.

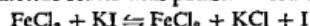
Quinine salicylate is precipitated, therefore follow *Method B for Precipitate-yielding Combinations*

## Incompatibility of Soluble Iodides

Iodides yield free iodine upon oxidation, and although iodine enters into many prescriptions it is usually assumed that the formation of free iodine in a mixture is unintentional, and steps should be taken to prevent it without altering the medicinal action of the mixture. If this is impracticable, the prescription should be referred back.

## 1 OXIDATION OF IODIDES WITH FERRIC SALTS

Ferric chloride reacts with potassium iodide thus—



There is no satisfactory method of correcting a mixture containing a ferric salt and an iodide. Correction could be effected by precipitating all the iron as hydroxide by the addition of ammonia, or by converting the ferric salt into a colloidal compound by adding an alkali citrate or tartrate (see below), but neither of these expedients should be used, the former yields an unsightly mixture, and the latter alters the composition. Hence the prescriber should be communicated with, and the substitution of the ferric salt by iron and ammonium citrate suggested. The latter is compatible with iodides.

Ferric chloride is compatible with iodides when an alkali citrate or tartrate is present, because the iron is converted to an organic compound which does not yield ferric ions. Exercise 16.5 is an illustration.

## 2 OXIDATION OF IODIDES WITH POTASSIUM CHLORATE

The following mixture is reputed to have been fatal—

*Recipe*—

Potassii Chloratis	ʒ i
Syrupi Ferri Iodidi	ʒ iss
Aquam	ad ʒ vi

## EXERCISE 16.5

*Recipe*—

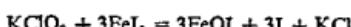
Liquoris Ferri Perchloridi B P C	1 ml
Potassii Iodidi	2 G
Potassii Citratis	4 G
Aquam	ad 60 ml

Fiat mixtura Signetur 15 ml bis in die sumenda

From the remarks above it follows that the ferric chloride must be converted to an organic compound before addition of the potassium iodide. Therefore dissolve the potassium citrate in about three-quarters of the vehicle, add the solution of Ferric Chloride and then the potassium iodide, finally adjust to volume.

The finished volume is green, due to the new compound formed by the ferric chloride and potassium citrate.

The probable reaction is—



The mixture is quite clear when freshly made, but deposits crystals of iodine upon standing for some time. The two reacting substances must be dispensed separately.

## 3 INCOMPATIBILITY OF QUININE BISULPHATE WITH SOLUBLE IODIDES

The following combination sometimes occurs—

*Recipe*—

Quininae Sulphatis	gr xxiv
Acidi Sulphurici Dilutis	ʒ i
Potassii Iodidi	ʒ ii
Aquam	ad ʒ vi

Fiat mixtura Signa Cochlearie amplum quartus horis sumendum

This is dispensed as follows—

The Dilute Sulphuric Acid is diluted to 3 oz and the quinine sulphate dissolved therein. The potassium iodide is dissolved in water, diluted to 3 oz, and mixed with the first portion.

The mixture is clear at first, but after about three days it may deposit bronze or olive green scales.

The reaction which takes place is the herapath' reaction for quinine. The sulphuric acid liberates hydroiodic acid from the potassium iodide, and the hydroiodic acid is partly oxidised by the sulphuric acid, yielding iodine. The iodine, hydroiodic acid and quinine sulphate then combine to form a compound called herapatite' or iodosulphate of quinine formula—



About 3 days' supply should be given to the patient where practicable otherwise the mixture

should be divided, sending the potassium iodide in one bottle, and the other ingredients in another

#### \* Incompatibility of Soluble Salicylates and Benzoates

##### 1 SOLUBLE SALICYLATES AND BENZOATES WITH ACIDS

Most acids decompose salicylates and benzoates, with formation of salicylic and benzoic acid respectively. The latter acids are only slightly soluble in water, and are therefore precipitated.

Incompatibility arising from the above combinations furnishes a useful illustration of the terms 'tolerated' and 'adjusted' incompatibility, mentioned on p 202

##### (a) Tolerated Incompatibility

Precipitation of salicylic or benzoic acid is tolerated when it is impracticable to prevent it without

altering the medicinal action of the mixture. Both acids are administered *per se*, and no untoward results are likely. The precipitated acids do not remain diffusible, hence *Method B for Precipitate Yielding Combinations* should be followed.

Exercise 16 6 is one in which precipitation of salicylic acid is usually tolerated.

##### (b) Adjusted Incompatibility

Precipitation of salicylic or benzoic acid is prevented when this can be effected without altering the action of the preparation. Exercises 16 7 and 16 8 are two common combinations in this class.

#### EXERCISE 16 6

##### *Recipe—*

Sodii Salicylatius	5 ii
Quinunæ Sulphatis	gr xii
Acidi Sulphurici Diluti	m. xxx
Aquam	ad 5 vi

Fiat mixtura Signa Cochlearia magna duo tertius horis sumenda  
Follow *Method B* (p 203) as indicated.

*Note* As will be seen below, precipitation of salicylic acid is prevented, when practicable, without appreciably altering the action of the mixture. In the above prescription it is probable that the sulphuric acid was included to dissolve the quinine sulphate, and that the prescriber overlooked the fact that the acid would decompose the sodium salicylate and prevent the formation of a clear mixture. Many pharmacists would take this view, and omit the acid.

#### EXERCISE 16 7

##### *Recipe—*

Sodii Salicylatius	5 G
Syrupi Limonis	20 ml
Aquam	ad 75 ml

Fiat mixtura Signetur 15 ml pro dose, more dicto sumenda

Lemon Syrup contains citric acid, and will therefore liberate salicylic acid from the sodium salicylate. Lemon Syrup is prescribed as a flavouring and may be replaced without altering the therapeutic action of the mixture.

Replace the Lemon Syrup with 19 ml of Syrup, and add 12 ml of Lemon Tincture.

Dispense the above prescription exactly as written, and note the precipitation of salicylic acid, then dispense with the suggested adjustment.

## EXERCISE 16 8

Recipe—

Sodii Salicylatus	gr xv
Caffeinae Citratus	gr x
Aquam	ad 3 i

Fiat haustus Sigma Statum sumat

Caffeine Citrate is a mixture of equal weights of caffeine and citric acid, damped and dried. There is an excess of citric acid over the quantity required in theory to form normal caffeine citrate. Further, caffeine is a weak base, consequently caffeine citrate prepared with molecular quantities of caffeine and citric acid would have an acid reaction. (Caffeine citrate is more soluble in water than caffeine, hence the use of the former.) With sodium salicylate and sodium iodide caffeine forms a very soluble compound,\* so that when caffeine citrate is prescribed with sodium salicylate or iodide, half as much caffeine should be substituted, and the mixture will be quite clear.

Dispense exactly as written, and note the precipitation of salicylic acid which ensues. Repeat, substituting 5 grains of caffeine for the citrate, and a clear mixture will be obtained.

\* Caffeine and Sodium Iodide B P C. is such a compound

## 2 SOLUBLE SALICYLATES AND BENZOATES WITH FERRIC SALTS

Ferric salts react with salicylates and benzoates to form ferric salicylate (violet) and ferric benzoate (buff-coloured). These compounds are insoluble in water, and are therefore precipitated.

Ferric benzoate is diffusible, hence *Method A for Precipitate-yielding Combinations* should be followed. Ferric salicylate, on the other hand, may not remain diffusible, hence *Method B should be followed*.

Exercise 16 9 is typical.

## Solubility of Ferric Salicylate

Ferric salicylate is soluble in solution of sodium or potassium bicarbonate, hence a clear mixture is formed if either is present.

Exercise 16 10 illustrates this fact.

## 3 SOLUBLE SALICYLATES WITH ALKALINE SUBSTANCES

The gastric juice contains hydrochloric acid, hence, after taking a dose of sodium salicylate a precipitate of salicylic acid is formed in the stomach. Although salicylic acid is occasionally administered *per se*

## EXERCISE 16 9

Recipe—

Liquoris Ferri Perchlорidi	5 1
Sodii Salicylatus	5 ii
Aquam	ad 3 vi

Fiat mixtura Sigma Coachlearre amphum ter die sumendum

Follow *Method B for Precipitate-yielding Combinations* (p 203)

## EXERCISE 16 10

Recipe—

Sodii Salicylatus	5 1
Sodii Bicarbonatis	5 ii
Liquoris Ferri Perchlорidi	m xv
Aquam	ad 3 vi

Dissolve the first two ingredients in about 4 oz of water in a 10-oz measure, add the Solution of Ferric Chloride, and notice the considerable effervescence. When effervescence has ceased, filter through cotton wool and pass sufficient water through the filtrate to produce 6 ounces.

it is too irritant to be generally tolerated. Hence, in prescribing sodium salicylate it is usual to order twice as much bicarbonate of soda as salicylate, thereby temporarily neutralising the gastric secretion and thus minimising formation of salicylic acid. Sodium salicylate in solution, and more especially in alkaline solution, absorbs oxygen, and the solution ultimately becomes brownish-black. Its efficacy is

not thereby impaired, but it is usual to darken the mixture *ab initio* by including Liquorice Liquid Extract of burnt sugar. When this is not done it is customary to warn the patient of the impending change in colour. The addition of 1 grain of sodium metabisulphite per ounce of mixture will considerably retard the change in colour, and it may be added with the prescriber's permission.

#### Incompatibility causing Evolution of Carbon Dioxide

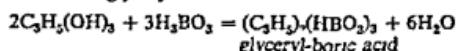
Four commonly-occurring combinations are—

##### 1 BORAX WITH SODIUM BICARBONATE AND GLYCERIN

In the presence of glycerin, borax decomposes to form sodium metaborate and boric acid—



The boric acid reacts with the glycerin to form a monobasic glyceryl boric acid—

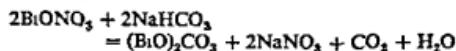


Glyceryl boric acid reacts with bicarbonates, liberating carbon dioxide. Boric acid does not liberate carbon dioxide from bicarbonates.

When these three substances are compounded they should be mixed with the water in an open vessel until effervescence ceases.

##### 2 BISMUTH SUBNITRATE AND SODIUM BICARBONATE

The reaction occurring when these are mixed with water is as follows—



The reaction proceeds slowly at ordinary temperatures, and if the mixture is dispensed in the ordinary way and bottled it is almost certain that sufficient carbon dioxide will be generated later to burst the bottle. The reaction should be accelerated by using hot water. No suspending agent is necessary, as bismuth carbonate is readily diffusible.

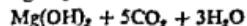
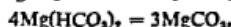
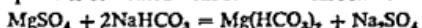
Bismuth Salicylate also reacts with sodium bicarbonate with formation of bismuth carbonate and evolution of carbon dioxide.

##### 3 AMMONIUM CARBONATE AND BICARBONATE WITH SYRUP OF SQUILL OR OXYMEL OF SQUILL

Both these preparations of Squill are acid. Tincture of Squill being the only B.P.C. preparation which is neutral. Substitution of the last named should be suggested to the prescriber, but if this course is not practicable the preparation should not be bottled until effervescence has ceased.

##### 4 SODIUM OR POTASSIUM BICARBONATE WITH SOLUBLE CALCIUM OR MAGNESIUM SALTS

The following reactions occur when magnesium sulphate is combined with sodium bicarbonate—



Similarly with soluble calcium salts, calcium bicarbonate is first formed, and then it gives up carbon dioxide to form calcium carbonate.

These reactions only slowly reach completion at ordinary temperatures, hence reaction should be accelerated by using the vehicle hot, and the preparation should be set aside for a short time for reaction to subside. No suspending agent is needed—magnesium carbonate and precipitated calcium carbonate being readily diffusible.

#### EXERCISE 16 11

#### *Recipe—*

Sodu Bicarbonatus	1 50
Boracis	1 50
Phenolis	0 75
Glycerini	25 00
Aquam	ad 100 00

Fiat nebula Mitte 3 : Signa More dicto utenda  
Follow the above directions

## Incompatibility of Iron Salts with Tannin

Iron salts react with tannin\* to form a greenish black or bluish black iron tannate. If the proportion of tannin is small, the mixture merely darkens in colour, but in larger proportion the iron tannate is

precipitated. The precipitate is usually diffusible, hence *Method A for Precipitate yielding Combinations*, should, when practicable, be followed.

Exercise 16.12 is typical

## EXERCISE 16.12

## Recipe—

Liquoris Ferri Perchloridi	5 i
Infusum Caryophylli	ad 3 iii
Fiat mustura Signetur Cochlearia modicum ex aqua ter in die	
sumendum	

## Incompatibilities of Liquorice Liquid Extract

Liquorice Liquid Extract owes its flavouring property to glycyrrhizin, a mixture of the calcium and potassium salts of glycyrrhizic acid. Acids decompose glycyrrhizin forming glycyrrhizinic acid, which is insoluble in water and is therefore precipitated. The precipitate rapidly clots to form a sticky black sediment which is difficult to diffuse. Further, because insoluble substances are tasteless, it follows that the flavouring property of the liquid extract is destroyed when the flavouring substance is rendered insoluble. Consequently Liquorice Liquid Extract

is useless as a flavouring agent for acid mixtures, and such prescriptions should, if possible, be referred back to the prescriber, for the flavouring agent to be changed.

Certain salts, e.g. calcium chloride, magnesium sulphate, tend to cause partial precipitation of glycyrrhizin. In these instances the precipitate, if any, remains diffusible. *Method A for Precipitate yielding Combinations* should be followed in order to minimise precipitation.

## INCOMPATIBILITIES OF SOLUBLE BARBITURATES

The alkyl and aryl derivatives of barbituric acid are nearly insoluble in water but their sodium salts are very soluble. Although generally prescribed in tablet form they are occasionally ordered in aqueous mixtures, and sometimes cause incompatibility. Even if no incompatibility occurs, aqueous mixtures of soluble barbiturates should be freshly prepared since hydrolysis occurs. In aqueous solution, phenobarbitone decomposes to phenylethylacetyl

urea. The following are examples of barbiturates used in the form of sodium salts—barbitone, phenobarbitone, amylobarbitone, pentobarbitone, hexobarbitone and quinalbarbitone.

The solutions are very alkaline and incompatibilities occur with ammonium salts and acids when the insoluble barbiturate may be precipitated. Other incompatibilities are usually due to a reduction of pH.

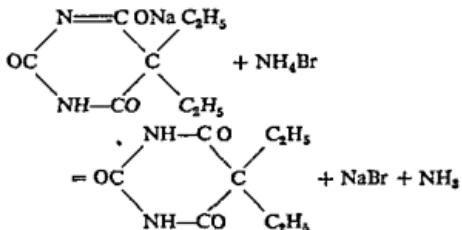
## Incompatibility of Soluble Barbiturates with Ammonium Bromide

When dissolved in water these compounds yield a solution which, in effect, is one of sodium hydroxide containing the barbituric acid derivative. The solution therefore reacts with ammonium bromide (see opposite column).

Hence, as shown in the equation, barbitone is formed, and being insoluble in water, it is precipitated—the precipitate being indissoluble.

When Soluble Barbitone or Soluble Phenobarbitone is combined with ammonium bromide, it may

be assumed that the prescriber intends the patient to receive a clear mixture. This may be produced by



\* A list of some commonly-used preparations containing tannin is given on p. 206.

replacing the ammonium bromide with sodium (or potassium) bromide. The qualitative action of these three bromides is the same, but quantitative action depends on the proportion of bromide radicle present, hence their bromide content must form the basis for substitution one for the other, as shown in Exercise 16.13

## EXERCISE 16.13

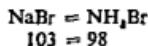
Recipe—

Phenobarbitoni Solubilis	gr x
Ammonii Bromidi	5 ii
Aquam	ad 3 iv

Fiat mustura. Signa Cochleare magnum nocte manque capendum.

First dispense as written, and observe the indissoluble precipitate of phenobarbitone

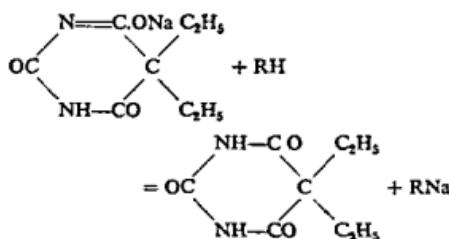
Repeat the exercise, substituting sodium bromide for ammonium bromide, thus



Therefore substitute  $\frac{120 \times 103}{98} = 126$  grains of sodium bromide for the 120 grains of ammonium bromide prescribed—the resulting mixture will be clear

## Incompatibility of Soluble Barbiturates with Acids

These substances are also incompatible with acids, and such incompatibilities are fairly common. In the case of Soluble Barbitone, for example, the sodium salt of the acid will be formed and barbitone precipitated. This may be expressed generally as follows (R = acid radicle)



Exercises 16.14 and 16.15 are typical examples, each containing an acid syrup. In each case follow Method B for Precipitate yielding Combinations, p. 203. If the prescriber's sanction can be obtained

it is better to substitute the insoluble barbiturate which is more stable in aqueous suspension.

Smith (1961) describes work done in the Pharmaceutical Society's Department of Pharmaceutical Sciences on prescriptions containing phenobarbitone sodium and amylobarbitone sodium. Tables 16.2 and 16.3 from this report gives the solubilities at varying pH and is a good guide to the possibility of precipitation.

The report also describes cases of soluble barbiturates prescribed with B.N.F. mixtures. For example,  $\frac{1}{2}$  grain of phenobarbitone sodium in  $\frac{1}{2}$  fluid ounce gave no precipitate with these mixtures.

Mist. pot. brom., Mist. pot. brom. et nux vom., Mist. pot. brom. et valerian and Mist. gent. alk.

On the other hand, amylobarbitone sodium in a concentration of  $\frac{1}{2}$  grain per  $\frac{1}{2}$  fluid ounce precipitated with all these mixtures except Mist. pot. brom. et nux vom.

In addition, Smith gives a list of some thirty salts and preparations likely to cause precipitation with soluble barbiturates.

## EXERCISE 16.14

Recipe—

Barbitoni Solubilis	gr 155
Syrupi Iodotannici	3 i
Aquam	ad 3 ii

Fiat mustura Mitte 3 i

Signa Cochleare medium hora somni sumendum

## EXERCISE 16.15

## Recipe—

Phenobarbitoni Solubilis	gr 1
Syrupi Glycerophosphatis	3 fl
Aquam Chloroformi	3 ss
Fiat mustura Mitte 3 ii	ad
Signa Cochlearium magnum nocte sumendum	

Table 16.2

Precipitation of Phenobarbitone from Mixtures

Concentration (gr/fl oz)	pH at which precipitation begins
½	6.6
1	7.4
1½	7.6
2	7.8
3	8.0
4	8.2
6	8.4
8	8.5

Table 16.3

Precipitation of Amylobarbitone from Mixtures

Concentration (gr/fl oz)	pH at which precipitation begins
½	7.8
1	8.6
1½	9.0
2	9.1
3	9.2
4	9.3
6	9.5
8	9.6

## Incompatibilities of Emulsifying Agents

The newer emulsifying agents present a number of difficulties which have been described under the individual substances in chapter 10. The following is a summary of the more important—

Methylcellulose— Phenol, chlorocresol, resorcinol, aminactine, ions such as mercury, zinc, aluminium, silver, and iron

Carboxymethyl cellulose— Phenol, chlorocresol, resorcinol, tannic acid, silver nitrate, phenylmercuric nitrate

Alkali metal Soaps— Cationic substances, mineral acids, di- and tri-valent metallic ions, high concentrations of electrolytes

Amine Soaps— Metallic Soaps— Emulsifying Wax—

Cetrimide Emulsifying Wax— Anionic substances, Cetomacrogol Emulsifying Wax

Cetromacrogol Emulsifying Wax— Quaternary ammonium compounds phenols, tannic acid

## REFERENCES

SMITH, G (1961) A Note on Soluble Barbiturates in Mixtures, *Pharm J* 187, 495-498

## REVISION EXERCISES 1 Recipe—

Cera Emulsif	8 per cent
Cetrimid	1 per cent
Paraff Moll Alb	25 per cent
Aquam	ad 100 per cent
Fiat cremor Mitte 3 ii	
Signa Frequenter utendus	

(continued overleaf)

## Revision Exercises continued

## 2 Recipe—

<i>Cov</i>	Bismuthi Subnitrat	3 i
	Sodu Bicarbonatus	3 ii
	Infusum Aurantii Recens	ad 3 iii
	Fiat mixtura. Signa Cochleare magnum quartu parte horae ante cibos capiendum.	

## 3 Recipe—

<i>1 per os</i>	Quininæ Sulphat	2 grm.
	Acdi Sulphurei Dilut	4 mil.
	Potassi Iodidi	4 grm.
	Aquam	ad 160 mil

Fiat mixtura. Signetur 8 mil. bis in die sumenda

## 4 Recipe—

	Theobrominæ et Sodu Salicylati	gr x
	Potassi Bicarbonatus	gr xv
	Aquam	ad 3 ss

Fiat mixtura Signa Ter die sumenda Mitte doses sex.

## 5 Recipe—

<i>Cov</i>	Magnesii Sulphat	3 iv
	Rhei pulverati	gr xx
	Sodu Bicarbonatus	3 iss
	Aquam Menthae Piperitæ	ad 3 iv

Fiat mixtura Signetur Cochleare amplum nocte maneque capiendum.

## 6 Recipe—

<i>Hd</i>	Phenobarb Sod	gr iss
	Syr Limon	gr 1
	Aquam	ad 3 ss

Fiat mixtura Mitte 3 viii

Signa Cochleare magnum nocte sumendum.

## 7 Recipe—

<i>Hd</i>	Sodu Benzoatis	
	Caffeina Citratus	ana
	Extracti Glycyrrhizæ Liquidi	12 mil
	Aquam	ad 90 mil.

Fiat mixtura Signetur 15 mil. pro dose more dicto sumenda

## 8 Recipe—

<i>Cov</i>	Glycerini Boracis	3 i
	Sodu Bicarbonatus	3 i
	Aquam	ad 3 vi

Fiat collunarium. Signa More dicto cum tanto aquæ calidæ adhibendum.

## 9 Prepare two ounces of the following cream—

Proflavine Hemisulphate	0 1 per cent
Aqueous Cream	to 100-0 per cent
Label The Antiseptic Cream	

## The Dispensing of Proprietaries

A FUNDAMENTAL principle of dispensing practice for the pharmacist is correctly to interpret the wishes of the prescriber. In the past there was little difficulty in conforming to this principle. With the remarkable increase in the prescribing of proprietaries a good deal of confusion which has given pharmacists much concern, has arisen. This is due to the fact that medical men are divided in their opinions as to how proprietary medicines should be presented, and in most cases give no specific directions in writing their prescriptions.

Two general methods are employed—

- 1 To remove all labels and other evidence of the source and nature of the product and apply the pharmacist's label which will carry his name and address, the prescriber's directions and the name of the patient. This presentation is, in fact, the traditional method and may involve transferring the product to one of the usual containers. This method would normally be followed in dispensing from bulk supplies of proprietary medicines.
- 2 To apply the pharmacist's label to an original pack without making any attempt to conceal the source and nature of the contents. The label would, of course, carry the same details as in the first method.

The argument for the first method is that the patient should not know the nature of the medicine. A little knowledge, it is said, is a dangerous thing and in this case may lead to self medication, or even to the patient advising his friends.

In favour of the second method it may be said that people today are more knowledgeable about things medical, and co-operate in the treatment much better if the doctor takes them into his confidence about the medicines he prescribes. Also, in cases of poisoning by overdosage or where a child obtains access to a potent drug, identification is immediate and the proper action can be taken.

It is certain that one method will not apply to all cases. It is the custom of some doctors to mark certain of their prescriptions N.P. (Nomen proprium) or O.P. (Original pack) to indicate that the contents should be fully disclosed. The Joint Formulary Committee of the British Medical Association and the Pharmaceutical Society has advised doctors to state the exact wording required and to avoid general terms which might be misleading.

In this country the traditional method is favoured and is usually employed unless directions to the contrary are given. The General Medical Services Committee of the British Medical Association have recently decided against the practice of always disclosing the nature of the medicine, unless the doctor directs otherwise. Many proprietaries are issued in bulk and in such cases are normally dispensed in the traditional manner. Buying in bulk is, of course, cheaper than buying in small individual packs, and this is favoured since it reduces National Health Service charges.

The student is advised to read the report of a discussion on this matter presented at the Pharmaceutical Conference 1958, and also a Draft Code on the Dispensing of Proprietaries published by the Pharmaceutical Society in 1961, which made the following general recommendations—

*Wrapping* All medicines should be wrapped in the traditional manner or, otherwise, supplied in a form of covering suitable for delivery.

*Labelling* All medicines should carry in an appropriate place a dispensing label.

*Original Packs* A prescription for an 'original pack' should be dispensed by supplying the product as received from the manufacturer with the addition of a dispensing label. Any literature supplied with the pack should be removed unless needed for the proper use of the preparation by the patient. It should be understood that, if there are special

circumstances, an 'original pack' may appropriately be supplied even if it is not prescribed

*Paper Bags and Skillets.* These should not be used

*Containers.* Unless reference is made to 'makers' container' it is implied that dispensing should be from bulk supplies and ordinary dispensing containers should be used. Care should be taken to select a container which, so far as practicable, carries out the intention of the maker, and regard should, therefore, be had to the type of container used for the original pack.

*Mixtures and other Fluid Preparations.* Containers should be plain or other suitably graduated bottles closed by corks or non metallic caps. Oral antibiotics and preparations in special dispensing packs should be supplied in makers' containers. If an oral antibiotic bears an instruction showing that the medicine should not be used after a certain date, this fact should be stated on the label.

*Tablets (Loose), Capsules, Pills.* Containers should be of glass, plastic or suitable metal or may be flanged boxes or card drums, when a less permanent type of container may properly be used. If two or more prescriptions for these preparations for the same person are dispensed at the same time, care should be taken in dispensing them to avoid the preparations being confused in use.

*Tablets (Foil or Strip Packing).* Containers should be boxes, tins or cartons. Dispensing envelopes may be used for small quantities.

*Lozenges.* Makers' containers should be supplied. If this is not practicable the containers used should be airtight.

*Cachets.* Containers should be boxes, tins or cartons.

*Eye Drops.* Makers' containers should be supplied or, if the pack is so designed that a carton or other outer covering virtually forms an integral part of it, the container may be supplied in such outer covering and the label be placed either on the container or on the outer covering, whichever is the most suitable place. Drop bottles may also be used.

*Nasal and Ear Drops.* Makers' containers should be supplied. If this is not practicable other appropriate containers should be used.

*Nebulisers.* Makers' containers should be supplied.

*Powders (Wrapped).* Slide boxes or tins should be used.

*Powders (Bulk).* Makers' containers should be used. If this is not practicable suitable rigid and powder-tight containers should be used.

*Ointments, Creams, Applications.* Makers' containers should be supplied. If this is not practicable, suitable non-absorbent containers should be used.

*Paints.* Makers' containers should be supplied. If this is not practicable, other suitable bottles should be used.

'Makers' container' is defined as a container and contents received from the manufacturer with any outer container or covering, literature, if any, and labels removed.

'Dispensing label' is defined as a label of the type traditionally used for dispensing, bearing the name and address of the supplier, the nature of the medicine and any other prescribed directions, the name of the patient and the date of dispensing.

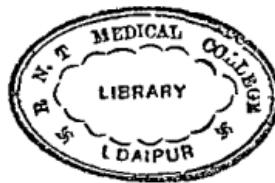
It will be obvious that there are many cases where the makers' container must be supplied. These will include injections packed in ampoules or multiple dose containers, nebulisers and other special products.

Any tendency towards a lax attitude to the dispensing of original packs must be avoided. There can be no alternative to the traditional idea that labelling, wrapping and other details of presentation should convey to the patient the conviction that care and accuracy have been employed in the preparation of his medicine.

A special point should be emphasised regarding the use of cardboard boxes and other non-permanent containers. These should be used only if the contents will not suffer damage or deterioration due to moisture, oxidation etc. The student is referred to a report of an investigation by Jolly and Benfield (1960) presented at an Evening Scientific Meeting of the Pharmaceutical Society on the use of paperboard containers for tablets.

#### REFERENCES

- THE BRITISH PHARMACEUTICAL CONFERENCE (1958)  
*Pharm J.*, 181, 254 'The Presentation of Dispensed Medicines'
- JOLLY, S. C. and BENFIELD, R. G. (1960) *ibid.*, 185, 469-473 'Paperboard Containers for Dispensed Tablets'
- 'DISPENSING OF PROPRIETARIES' (1961) *ibid.*, 186, 275



## Medical Gases

THE following is a brief account of the medical gases in common use together with some details of their containers storage and simple accessories

### CARBON DIOXIDE ( $\text{CO}_2$ )

Carbon dioxide is administered to stimulate respiration in emergencies, for example in cases of drowning or in poisoning by carbon monoxide, morphine or depressants, and, to some small extent, therapeutically in concentrations of 5 to 10 per cent in oxygen. Previously used to improve respiration generally it is now reserved mainly for emergencies

### CYCLOPROPANE ( $\text{C}_3\text{H}_8$ )

This is a powerful anaesthetic. Twenty per cent in oxygen produces anaesthesia for surgical purposes, 8 per cent produces light anaesthesia, and 4 per cent analgesia. Because of its explosive properties it is usually administered in a closed circuit system

### ETHYLENE ( $\text{C}_2\text{H}_4$ ), not B.P.

This has now been replaced almost entirely by cyclopropane

### NITROUS OXIDE ( $\text{N}_2\text{O}$ )

This is the oldest, safest and most common gas used in dental and obstetric practice to produce anaesthesia or analgesia by inhalation. It is used in mixtures containing not less than 12 per cent with air, or with air and 10 to 15 per cent of oxygen. Anaesthesia can be induced by nitrous oxide alone, but for extended periods an addition of 10 to 15 per cent of oxygen may be employed

### OXYGEN ( $\text{O}_2$ )

During anaesthesia oxygen is necessary, just as it is in the conscious state. It may be necessary in such conditions as lung or heart diseases, in asphyxia or poisoning by cerebral depressants and haemolytic

poisons. For inhalation over extended periods 50 to 60 per cent is used. Higher concentrations may prove dangerous except at high altitudes when as much as 80 per cent may be necessary. The addition of 5 to 10 per cent of carbon dioxide to oxygen induces deep breathing, and is used when respiratory stimulation is necessary and in carbon monoxide poisoning

### HELIUM (He)

Because of its low molecular weight compared with oxygen, mixtures of helium with air, oxygen or anaesthetic gases make breathing easier, and this may prove of great value where there is mechanical or pathological obstruction. An air-helium mixture containing 80 per cent of helium may be used for resistant asthmatic attacks

### SAFETY PRECAUTIONS

Medical gases are stored in steel cylinders which are made to withstand a pressure of 3,000 lb/in<sup>2</sup>. No gas is stored at a pressure greater than 2,000 lb/in<sup>2</sup>, therefore, there is a safety factor of 1,000 lb/in<sup>2</sup>.

The cylinders are similar in appearance, and the possibility of confusion introduces a serious risk that the wrong gas might be administered. To avoid this a British Standard Specification and Code of Practice (1955), prepared for the guidance of those who handle and use medical gases, gives recommendations on the care and maintenance of cylinders and anaesthetic equipment.

Each type of gas is given a colour coding which readily identifies the cylinder, which should also bear the chemical symbol or name of the gas (see Table 18 i).

The possibility of fire or explosion must be guarded against, and a report was prepared in this connexion by a Working Party appointed by the

Table 18 I

Medical Gases  
Cylinder Contents and Colour Coding

Gas	Contents of cylinders	Colour code
Carbon Dioxide	1, 2, 4, 7 and 14 lb	Grey
Cyclopropane	8, 20, 40 and 80 gal	Orange
Helium	22 ft <sup>3</sup>	Brown
Nitrous Oxide	25, 50, 100, 200, 400, 800 and 2,000 gal	Blue
Oxygen	18, 36 and 72 gal 24, 48, 120 and 180 ft <sup>3</sup>	Black body White top
Oxygen and Carbon Dioxide mixtures	18, 36 and 72 gal 24, 48 and 120 ft <sup>3</sup>	Black body Grey and white top
Helium and Oxygen mixture 79%–21%	22 ft <sup>3</sup>	Black body Brown and white top

### Checking Cylinder Contents

This is done by measuring the pressure with a pressure gauge, or by weighing. Nitrous oxide, ethylene and cyclopropane are in liquid form in the cylinders, and if the gas is withdrawn slowly the pressure may remain constant until most of the contents have been released. In such cases weighing is the only reliable method of checking the contents. For this purpose the weight of the empty cylinder is stamped on the outside.

Pressure gauges for oxygen are calibrated in lb/in<sup>2</sup>, 2,000 lb/in<sup>2</sup> indicates a full cylinder, and the gauge is also marked to show when the cylinder is  $\frac{1}{4}$ ,  $\frac{1}{2}$  and  $\frac{3}{4}$  full. In smaller outfits for domiciliary use the gauge is marked 'Full', 'Half' and 'Refill'.

### Care of Cylinders

Recommendations for the storage and care of cylinders of medical gases is made in British Standard 1319:1955, already mentioned. The cylinders should be stored in a cool, well-ventilated room free from material of an inflammable nature. The room should be large enough for proper grouping of cylinders according to their contents, hence avoiding confusion. A special rack is recommended for cylinders, illustrated in an Appendix to the Standard, which is aimed at ensuring that the cylinders will be used in rotation according to whether they are new or older stock.

### Outlet Valves

Because of the pressure in the cylinders the release of gas for use must be carefully controlled, and various outlet valves are used for this purpose. The latest type of outlet valve eliminates the possibility of using the wrong cylinder since only the correct cylinder will fit the attachment to the anaesthetic apparatus. This is the non-interchangeable flush type (pin index) valve, which fits into a yoke on the anaesthetic apparatus. The yoke has pins which fit into holes in the valve. The position of the pins and holes are different for each type of gas cylinder, so that a mistake is impossible. Fig. 18 I illustrates

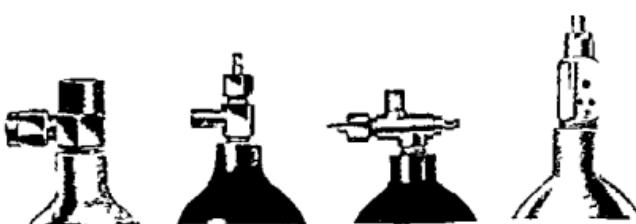


Fig. 18 I MEDICAL GAS CYLINDER VALVES

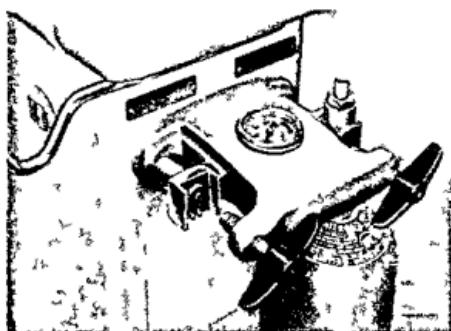
Outlet Valves Left to right  
bull nosed, straight type 7  
angled type 8, flush type  
(pin index)

(Courtesy British Oxygen Co. Ltd.)

Ministry of Health (1956), which includes a Safety Code dealing with explosive medical gases. Danger from fire or explosion may arise from electrical apparatus in general and, in particular, apparatus used for radiography and diathermy in hospitals.

Grit in outlet valves can be a cause of trouble since, if it gains access to the reducing valve mechanism, there is a possibility of ignition of inflammable gases by friction. Such grit may be blown out of the valve by opening it slightly before the cylinder is used.

Oil and grease may ignite spontaneously in presence of high pressure oxygen. Such lubricants must, therefore, not be used with cylinder valves or attached mechanisms.



*Fig 18.2 FLUSH TYPE VALVE YOKE, SHOWING CYLINDER IN POSITION  
(Courtesy British Oxygen Co Ltd)*

this type of outlet valve, which is likely to supersede all the others, and the three other types which are the bull nosed, the straight type 7 and the angled type 8. Fig 18.2 shows a cylinder with the flush type valve fitted to the yoke in the anaesthetic equipment.

It should be realised that the outlet valves mentioned above are intended to open and close only the outlet from the cylinder. To control the rate of flow, fine adjustment valves and regulators are used.

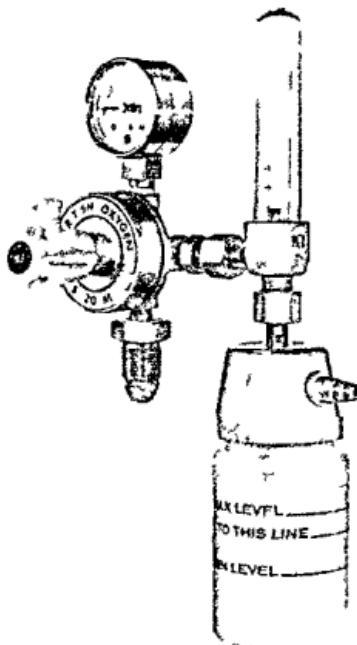
#### Fine Adjustment Valves

These are relatively simple, consisting of a tapered needle which moves through an orifice, its position being controlled by a small hand wheel. The valve spindle connecting the wheel with the needle valve has a relatively fine pitched thread giving fine control.

It has two disadvantages. First, if there should be an obstruction in the breathing apparatus, which is fed from the cylinder, pressure will build up and the connecting tubing will burst. Secondly, with compressed gases such as oxygen as cylinder pressure falls the flow rate diminishes and has to be controlled by hand to maintain the flow at constant level.

#### Regulators

These do not suffer the disadvantages of the fine-adjustment valves since excess pressure cannot build up on the outlet side. If pressure in the tube connected to the breathing apparatus rises too much a back pressure closes the valve in the regulator and cuts off the supply of gas. Fig 18.3



*Fig 18.3 REGULATOR, FLOWMETER, AND HUMIDIFIER*

*(Courtesy British Oxygen Co Ltd)*

#### Flowmeters

The rate of flow of gas to the patient is judged in various ways. On occasion it may not be measured but merely judged by observations on a rebreathing bag connected to the face mask. It is however, advisable to measure the flow accurately, and this may be done by different types of apparatus, e.g. the fixed orifice and variable orifice types. In the former the gas flows through a fixed orifice and the gas pressure is measured on a dial reading pressure gauge behind the orifice. The flow is commonly recorded in litres per minute.

In one variable orifice type the gas passes up a calibrated vertical tube with a conical bore containing a small, light weight bobbin. The gas passes between the bobbin and the inside wall of the tube. Depending on the pressure of the gas flow, the bobbin will rise in the tube to a point where equilibrium is established between the pressure of the gas and the weight of the bobbin. Grooves in the

rim of the bobbin cause it to revolve on its vertical axis in the upward flow of the gas. This keeps the bobbin in a central position, thus preventing friction. Flow rate is indicated by the height of the bobbin as noted on the graduated scale, Fig. 18.3

#### Humidifier

This is a container which is connected to the flow-meter unit, Fig. 18.3, and is filled to a marked level with water. A safety valve is incorporated to avoid a build up of excess pressure inside the unit.

### OXYGEN INHALERS

#### The Disposable Inhaler

This is made of a light plastic material and can be made to fit the shape of the face, giving a good seal and a considerable degree of comfort over a prolonged period. The wearer can converse freely without difficulty while wearing it. Since it is used only by one patient and then discarded there is no risk of transmitting infection to others. Fig. 18.4 shows the British Oxygen 'Polymask' disposable oxygen inhaler.

#### The Nasal Inhaler

This type covers the nose only. Two tubes from the nose-piece pass round each side of the mouth and join at the chin to form one tube. This leaves the mouth free so that the patient can eat and talk. From the chin a plastic connecting tube leads to a reservoir rebreathing bag. Oxygen enters at a point on the plastic connecting tube and is led to the base



Fig. 18.4 THE POLYMASK DISPOSABLE INHALER  
(Courtesy British Oxygen Co. Ltd)



Fig. 18.5 THE NASAL INHALER  
(Courtesy British Oxygen Co. Ltd)

of the reservoir where it is released. In the nose-piece there is a valve to allow inhalation and exhalation, Fig. 18.5.

This is a form of the so called B.L.B. Inhaler named after the doctors who introduced it, Boothby, Lovelace and Bulbulian (1938).

#### The Oronasal Inhaler

This type covers both nose and mouth otherwise it is similar to the Nasal Inhaler described above. It is particularly useful if nasal breathing is poor, and for resuscitation, Fig. 18.6.

In use, the valve in the nose-piece which leads to the atmosphere, cuts down the escape of exhaled air until the reservoir is full and also cuts down the intake of air while there is sufficient gas in the reservoir.

The reservoir will deflate and expand as the patient inhales and exhales. At the beginning of exhalation the breath is richest in oxygen, and this will return to fill the deflated reservoir, along with fresh oxygen from the cylinder. The rest of the exhalation will consist largely of carbon dioxide and will be expelled through the nose-piece valve to the atmosphere.



*Fig 186 THE ORONASAL INHALER  
(Courtesy British Oxygen Co Ltd)*

To obtain a high concentration of oxygen the flow should be such that the reservoir does not completely deflate at the end of each inhalation and to obtain a less-rich supply the rate of flow is reduced, the reservoir collapsing before the end of the inhalation, and air then being drawn in through the nose-piece valve.

If excess moisture should collect in the reservoir it can be drained away through the opening at the base

### The Nasal Catheter Set

This consists of a Tudor Edwards spectacle frame with two nasal catheters and a combined flowmeter/humidifier. The spectacle frame has tubes below the eye pieces carrying two intranasal catheters, which are pieces of cycle valve tubing. The oxygen supply tube divides into two by a Y piece, the tubes going one to each side of the frame and bending downwards towards the nostrils.

The oxygen must be humidified to avoid pain due to the effect of the dry gas on a limited area of mucous membrane. The tube which bubbles the oxygen through the water in the humidifier may act as a flowmeter. For this purpose it has a number of vertical holes and an accompanying scale. The pressure of gas is determined by the lowest hole from which gas escapes and is read from the corresponding graduation on the scale.

The Drug Tariff specifies a Lightweight Single Unit Set and a Multiple Unit Set which may be supplied under the National Health Service for oxygen therapy.

The lightweight set is supplied in a box, with full instructions for domiciliary use. It consists of the necessary kit for use with a 4 ft<sup>3</sup> cylinder (bull nosed valve). The kit includes a reducing valve giving pressures of 50, 40 and 7 lb/in<sup>2</sup>, a contents gauge, a two-flow selector cap marked at 'Med' or 'High', corresponding to a flow rate of 2 or 4 litres per minute, and two disposable inhalers.

The multiple unit set comprises a fine adjustment valve giving a flow rate of 1 to 6 litres/min a contents gauge with  $\frac{1}{4}$ ,  $\frac{1}{2}$  and full markings, a flowmeter of the bobbin type, and two disposable masks.

If specially ordered with this set a B.L.B mask set or a Tudor Edwards catheter set may be supplied. In the latter case a flowmeter/humidifier is supplied in place of the bobbin type flowmeter.

---

## Part Two Sterilisation Practice

---

19

### Microbiological Aspects of Sterilisation Processes

#### *Introduction*

THE parenteral administration of drugs in medicine and the use of aseptic technique in surgery have made the preparation and storage of sterile medicaments, materials and equipment of great importance to the pharmacist. A sterile preparation is one that is free from living micro-organisms. Bacteria, bacterial spores, yeasts and mould spores may be contaminants of medicaments but most sterilisation problems are caused by bacteria and, particularly, their spores.

Bacteria are minute organisms that are probably more closely related to the fungi than to any other plant or animal group and, consequently, they have been made a class of the Thallophyta in the vegetable kingdom. Until recently they were believed to be unicellular, to have no true nucleus, and to multiply only by simple fission, but modern techniques have demonstrated cross-walls, nuclei and complex life cycles (Bissett, 1962, 1955). Bacteria are ubiquitously distributed over the surface of the earth, being

found in the atmosphere, in soil and water and on the skin. Many are beneficial (e.g. some promote the decay of organic matter in the soil, others fix nitrogen and a number are used in industrial processes) but others cause disease. The latter are called pathogenic bacteria or pathogens, the term non-pathogenic being applied to those organisms that are not known to cause disease. Micro-organisms that live in or on a part of the body without producing harmful effects are called commensals but an organism that is a commensal in one region may be pathogenic elsewhere, e.g. *Escherichia coli* is non-pathogenic in the intestine but in the urinary and gall bladders it causes severe inflammation. Therefore, it will be realised that only sterile preparations are safe for injection into the body. Medicaments prepared by the usual methods of dispensing will not be safe due to the risk of infection from any pathogenic organisms present. This is highly probable in certain substances which, by virtue of their sources, may be expected to be heavily contaminated, often with disease-producing bacteria.

#### BACTERIAL SPORES

When bacteria reproduce by fission each cell divides to form two daughter cells. Under very favourable conditions this may take place every 20 minutes, one cell thus giving rise to several millions in 24 hours. In certain circumstances some bacteria, but by no means all, produce a resting form called a spore.

Spore formation begins with a localised concentration of nuclear material, this becomes surrounded by a wall and then the mother cell degenerates. Meanwhile most of the water inside the spore is taken up ('bound') by the colloidal constituents of its protoplasm. Respiration of the protoplasm, or sporoplasm as it is now called, is slowed down and in this state the bacterium is able to live for a long time under adverse conditions.

Spore production may be caused by drying, exhaustion of nutrients or the accumulation of harmful products of growth, but it is not always due to an unfavourable environment (Salle, 1961, Williams, 1952) and, therefore, the suggestion has been made (Bissett, 1950) that the spore is a stage in the life cycle of sporing bacteria adapted by its small size to dispersal by air currents.

Spores are very much more resistant to high temperatures and dessication than ordinary vegetative cells and they are more resistant to the action of chemicals. These resistant qualities may be due in part to the fact that most of the spore water is 'bound' and, since in this form it cannot readily take part in chemical reactions, the destructive processes

involved in sterilisation by heat and chemicals are retarded. This is illustrated by the spores of *Bacillus subtilis* which can withstand boiling for three hours, although the vegetative cells of the same organism are destroyed in 20 minutes.

Many alternative explanations of spore resistance have been offered, including the high content of lipid (Sugiyama, 1951), calcium (Curran, 1952) and dipicolinic acid (Powell and Strange, 1956) which,

separately or together, are believed to form heat-stable complexes with proteins.

In conditions favourable to growth the spore reverts to the vegetative state. Moisture is taken in, the sporoplasm swells and a rupture appears in the spore coat through which the sporoplasm is extruded. The old spore wall is gradually thrown off and the vegetative cell thus formed begins to multiply once more.

## FACTORS AFFECTING GROWTH OF BACTERIA

The following are the chief factors affecting the growth of bacteria.

### 1 NUTRITION

All bacteria need mineral salts and sources of carbon and nitrogen. Some, like the denitrifying bacteria, can use very simple materials, e.g. carbon dioxide as the source of carbon and ammonium salts or nitrates as the source of nitrogen. Most, including the pathogens, must be provided with much more elaborate compounds, e.g. carbohydrates or organic acids as carbon sources and proteins or, more often, their degradation products as sources of nitrogen.

The synthesis of bacterial protoplasm is the result of chains of chemical reactions. Certain compounds, known as essential metabolites, form vital links in these chains and, therefore, growth stops if they are not available. As some bacteria cannot synthesise these substances they must be included in the growth medium, when they are called growth factors. Important examples are para-amino-benzoic acid (see p. 455), cyanocobalamin (vitamin  $B_{12}$ ), aneurine hydrochloride (vitamin  $B_1$ ) and folic acid.

It follows that to produce vigorous growth of bacteria, suitable and adequate sources of carbon, nitrogen, mineral salts and growth factors must be present in the culture medium (see 'The preparation of culture media' and 'Sterility testing').

However, some bacteria can multiply when the concentration of nutrients is extremely low. For example, growth can occur in distilled water, a fact having an important bearing on the preparation of solutions for injection (see 'Water for Injection').

### 2 MOISTURE

Bacteria require moisture in order to utilise the food substances mentioned above. Usually a medium for the growth of bacteria must contain at least 20 per cent of water. In the absence of moisture bacteria cease to multiply but spore-bearing forms may continue to exist in spore form, sometimes for many years.

### 3 AIR

Some bacteria will grow in air and are called aerobes, e.g. *Pseudomonas aeruginosa*, others can multiply only in the absence of oxygen and are known as anaerobes, e.g. *Clostridium tetani*. A third group, facultative anaerobes, are able to grow with or without air, e.g. *Escherichia coli*. Exact classification of bacteria according to their oxygen requirements is complicated by the fact that the cultural and metabolic behaviours of an organism to oxygen may differ. For example, it may grow well on the surface of a solid culture medium in air without using oxygen in its metabolism. For a detailed consideration of this difficulty see McBee *et al.*, 1955.

Anaerobic bacteria may be successfully cultivated by providing an oxygen free atmosphere or by adding reducing substances to the growth media.

The state of oxidation or reduction of a medium can be measured and is known as the oxidation-reduction potential (see Cooper and Gunn, 1957a).

### 4 TEMPERATURE

Most pathogenic bacteria multiply best at 37°C, and the sterility tests of the *British Pharmacopœia* prior to 1963 were incubated at this temperature. However, because some common and serious contaminants of wounds, eye drops and injections (e.g. *Pseudomonas spp.*) have an optimum growth temperature of about 30°C and may not be detected in tests performed at 37°C the possibility of using a lower temperature was investigated. After Pittman (1946) had shown that all contaminants obtained from a series of tests at 37°C would also grow at 31°C the *United States Pharmacopœia* adopted 30 to 31°C and the *British Pharmacopœia* has now followed suit.

Some saprophytes have an optimum range of 55 to 80°C and are known as *thermophiles* to distinguish them from *mesophiles*, the group that includes pathogens, for which the optimum ranges from 20 to 45°C. Among the thermophiles are bacteria that cause spoilage of canned foods if inadequate heat

treatment is given during processing, the spores of some species, e.g. *Bacillus stearothermophilus*, are extremely heat resistant and are used to test the efficiency of heat sterilisation processes.

At temperatures approaching 0°C most organisms stop multiplying, but they remain alive, and this behaviour is utilised in the preservation of cultures of micro-organisms by freeze-drying. Unfortunately, a few bacteria, called *psychrophiles*, can multiply at low temperatures and, if present as contaminants, may damage blood and other nutritive materials that require storage in a refrigerator (Annotation 1959).

Temperatures above 50°C are harmful, particularly if moisture is present. All vegetative cells are killed by exposure to dry heat at 100°C for 1½ hours or moist heat at 80°C for an hour. Spores are more resistant, and comparable lethal exposures are dry heat for one hour at 150°C and moist heat for half an hour at 115°C, (see also pp 309, 316).

#### 5 pH

The optimum pH for growth is about 7.4, although this varies with different organisms. Growth is less rapid as the reaction of the liquid is made more acid or alkaline. Solutions that are strongly acid or alkaline are bactericidal, a fact of some importance in sterilisation (p. 317).

#### 6 LIGHT

Exposure to sunlight in the presence of air has a harmful action on bacteria and may inhibit growth or destroy the organism. It is for this reason that incubators used for growing bacteria have no windows. The damage is caused chiefly by light waves from the ultra-violet region and this explains the occasional use of ultra-violet lamps for reducing the contamination of atmospheres and surfaces.

Apart from its action on bacteria, light may produce changes in the medium in which they are growing, rendering it unable to support growth, (see 'Ultra-violet light'). Hence it is important to store culture media in a dark place and to use it as soon as possible.

#### 7 OSMOTIC PRESSURE

Bacteria behave rather sluggishly to changes in osmotic pressure but they are plasmolysed by strongly hypertonic solutions and swell, and may burst if the surrounding medium is hypotonic (see 'Isotonic solutions'). Suspensions of bacteria for test purposes should be in diluents of optimum osmotic pressure, and the inhibitory effect of strongly hypertonic solutions must be remembered in sterility testing (q.v.).

#### 8 SUBSTANCES INIMICAL TO GROWTH

Many substances can inhibit the growth of bacteria. Substances that prevent the growth of bacteria without destroying them are called *bacteriostats* while substances that kill bacteria are called *bactericides*. However, substances can be bacteriostatic in low, and bactericidal in high concentrations, and bacteria may die if subjected to prolonged bacteriostasis.

Bactericides are extensively used in the official injections,

(a) As preservatives, to kill vegetative bacteria and prevent the development of spores accidentally introduced into multiple dose containers during the withdrawal of the successive doses (see chapter 20).

(b) In the sterilisation method known as Heating with a Bactericide to kill vegetative bacteria and spores. Destruction of the latter is made possible by a higher concentration of bactericide and a raised temperature (98 to 100°C) (see chapter 22).

#### Application to Sterility Testing

Consideration of the above factors is necessary to establish the most suitable conditions for the growth of bacteria. Thus, to produce the rapid and luxuriant growth required, for instance, in the preparation of bacterial cultures or in testing for sterility, it is necessary to provide ample food material, sufficient moisture and a suitable hydrogen ion concentration, and to maintain the temperature at the optimum by means of an incubator that will also exclude light. For anaerobes precautions must be taken to ensure a low oxidation reduction potential. In all cases the presence of excessive quantities of substances having bacteriostatic or bactericidal action must be avoided.

#### Application to Preservation of Injections

A study of these factors will also indicate the most favourable conditions for bacterial growth. Multiplication of organisms may be prevented by maintaining complete dryness or, in the case of liquids, by cold storage, by adjustment of the reaction to a pH value unsuitable for growth or by the addition of a bactericide. With solutions to be injected it is not usually possible to control all these factors and the course normally adopted to prevent multiplication of organisms inadvertently introduced during use is the addition of a bactericide. However, it should be borne in mind that the medicament itself may, in some cases, produce a solution having a hydrogen ion concentration unfavourable to the growth of bacteria.

## PHASES OF BACTERIAL GROWTH

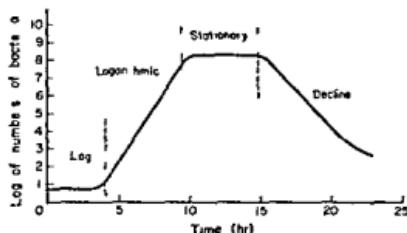


Fig. 19.1 TYPICAL GROWTH CURVE

Four distinct phases of growth are exhibited when bacteria are freshly inoculated into a satisfactory liquid medium and incubated under optimal conditions (Fig. 19.1)

### 1 LAG PHASE

Immediately after inoculation there is an interval of rest during which the bacteria seem to rejuvenate themselves. This is followed by a period of considerable growth activity in which, although there is no cell division, the cells increase in size and metabolism is very high. Towards the end of the phase multiplication begins and rapidly increases in rate.

This phase usually lasts two to three hours but its length is affected by a number of factors of which the following are of particular importance in sterility testing:

#### (a) Inoculum Size

The smaller the inoculum, the longer the lag. It appears that before an organism can make use of certain nutrients in a culture medium it must convert them into more suitable forms, and it does this by liberating enzymes. If the number of cells in the inoculum is very small these enzymes, together with the products of their activity, may be rapidly dispersed and diluted by diffusion, then the bacteria fail to multiply and the lag phase is lengthened and may even be indefinitely prolonged. On the other hand, if a large inoculum is transferred the necessary concentration of suitable metabolites is quickly built up, this is partly because so many organisms are contributing, partly because the correspondingly larger volume of medium, inoculated at the same time, already contains significant amounts of these metabolites and partly because more are released by the breakdown of the appreciable number of dead bacteria in the inoculum. As a result the phase is shortened.

Pharmaceutical products are tested for sterility by incubating samples in culture media. Only rarely will these samples be heavily contaminated and, for the reason just explained, there is a danger that the small numbers of bacteria present may not multiply sufficiently to produce the detectable turbidity used as an indicator of contamination. Consequently, the amount of preparation or material taken for the test should be as large as other considerations (see 'Sterility testing') allow, to ensure that as many organisms as possible are transferred to the culture media.

#### (b) Sensitivity of Medium

Some culture media, because they contain nutrients that are very easily metabolised by bacteria, are more sensitive than others, i.e. they reduce the lag phase by rapidly promoting the growth of small inocula. Such media are essential for sterility testing and before a choice is made tests for sensitivity should be carried out using small inocula of organisms that are demanding in their nutritional requirements (see p. 233).

#### (c) Previous History of Organism

Bacteria that were rapidly multiplying under ideal conditions when transferred from their previous medium will have a short lag, but organisms suffering from the toxic effects of a previous poor environment take longer to recover, and a prolonged lag results.

Often, bacteria in pharmaceutical preparations have been subjected to heat treatment or to long contact with a bactericide or a solution of unfavourable pH and, therefore, they may show a very long lag time even in optimal test media. It is for this reason that the *British Pharmacopœia* directs the long incubation period of 7 days for sterility tests.

### 2 LOGARITHMIC PHASE

This follows the lag phase and usually lasts about 6 hours. During this period growth is at its maximum and the number of bacteria increases logarithmically, i.e. the graph obtained by plotting the logarithm of the number of bacteria per millilitre against the time is a straight line. The number of organisms required to render the medium turbid varies slightly with the size of the organisms but turbidity can usually be detected when about 100 million organisms per ml are present and is quite marked with 1,000 million or more organisms per ml. The time at which detectable turbidity is produced depends upon the rate of multiplication of the

cells. Although this is governed by the type of organism it also depends to a marked extent on the suitability of the medium and the incubation temperature, both must be carefully chosen in sterility testing.

### 3 STATIONARY PHASE

In this phase division is slower, reaching a point where the number of new bacteria formed is approximately balanced by the number dying. The reasons for the decreased reproduction rate are not known with certainty but the main factors appear to be exhaustion of readily utilisable nutrients, accumulation of toxic by-products of growth and, in the case of aerobes, deficiency of oxygen.

## PREPARATION OF CULTURE MEDIA

Consideration of the factors that affect the growth of bacteria has shown that a satisfactory culture medium must have

- 1 Sufficient moisture
- 2 Adequate nutrients
- 3 The optimum pH range
- 4 A suitable oxidation reduction potential.

In addition it must be sterile and its sterility preserved by storage in adequately closed containers.

In the selection and preparation of media for use in the sterility tests of the *British Pharmacopoeia*, very careful attention should be given to all these points.

### 1 MOISTURE CONTENT

All culture media, liquid and solid are formulated to contain plenty of water.

Water that has been purified by distillation or by ion exchange is preferred to tap water because the latter is of uncertain composition and, during sterilisation, its calcium and magnesium salts give heavy precipitates with the phosphates in meat extracts and peptones.

Particular care is necessary to avoid contamination with copper, traces of which strongly inhibit bacterial growth. Distilled water should be obtained from a heavily tinned or, preferably, an all glass still.

### 2. NUTRIENTS

#### (a) Nitrogen Sources

Originally, watery extracts of fresh meat, e.g. Bouillon (infusion broth), were used to supply all the nitrogenous requirements of bacteria but, later,

### 4 DECLINE PHASE

The stationary phase passes into one in which the number of organisms dying increasingly outnumbers the newly-formed bacteria, division ultimately ceases and the culture becomes sterile. The time taken for all the organisms to die depends primarily on the toxicity of the by-products of their growth. It may be only two or three days but often it is several months.

(More extensive treatments of this important subject will be found in Hinshelwood (1946), Oginsky and Umbreit (1955), Porter (1946), Werkman and Wilson (1951) and Wilson and Miles (1955).)

## CULTURE MEDIA

fresh meat was replaced by commercial meat extracts, such as Lab Lemco, because the resulting broths (Meat extract broths) were easier to prepare, more reproducible and cheaper.

Both infusion and meat extract broths contain almost no protein and owe their growth promoting qualities to small amounts of nitrogenous muscle extractives, mineral salts and sugars. Because of the heat used in preparing the infusions and concentrating the extracts certain important nutrients are destroyed.

A considerable advance was made with the discovery that the infusions and extracts could be partially or, sometimes, completely replaced by peptones. These are breakdown products of animal or plant proteins and are obtained from the latter by digestion with suitable enzymes, usually papain, trypsin or pepsin. They contain a variable mixture of protein degradation products which, in order of complexity, include proteoses, polypeptides and amino-acids.

Since bacteria rarely attack natural proteins but readily metabolise the less complex breakdown products, most peptones are prepared to contain only small amounts of proteoses and the larger polypeptides. Often, the protein source greatly influences the suitability of the peptone, e.g. peptone from casein is rich in growth factors and amino-acids, as the latter are very simple compounds they pass easily through the bacterial wall and are readily used for cell growth.

Peptones are water soluble, not coagulated by heat (unlike most natural proteins) and, because of their amphoteric nature, are good buffers. All these properties are most valuable in an ingredient of a culture medium.

A particularly useful peptone is produced by the digestion of fresh meat. The so-called digest broths, made in this way, have exceptionally good growth-promoting qualities.

#### (b) Carbohydrate Sources

Carbohydrates are readily utilisable sources of energy. For most bacteria, adequate amounts are provided by meat extracts and digests and only occasionally is it necessary to add a carbohydrate supplement such as dextrose.

#### (c) Mineral Salts

The materials used as nitrogen sources also supply the mineral requirements of most organisms. It is customary to add about 0.5 per cent of sodium chloride to most culture media and this, together with the other constituent, provides a suitable osmotic pressure.

#### (d) Growth Factors

Peptones, meat extracts and certain meat infusions provide growth factors but, unless they are used in fairly high concentrations, the amounts of some of the important vitamins of the B complex may be inadequate for the rapid growth of small inocula. As an alternative to these high concentrations, yeast extract, which is a very rich source of the B vitamins, may be added as a growth factor supplement.

(For further information see Difco Manual (1953) and Oxoid Manual (1961).)

#### 3 pH RANGE

Because most pathogenic bacteria grow best at a pH of about 7.4 the B.P. specifies that, except where otherwise stated, the post sterilisation reaction of the culture media used in sterility testing must lie within the range 7.2 to 7.8.

The unadjusted media are usually acid, and adjustment is carried out with standardised volumetric solutions of sodium hydroxide; it can be followed and checked either electrometrically, using a pH meter, or colorimetrically, using a pH indicator and colour standards.

The colorimetric method is popular because the apparatus is inexpensive and the technique is convenient and simple. Essentially it consists of adding an indicator to a known volume of the culture medium and titrating until the colour is between those of two solutions containing the same indicator and buffered to a pH on either side of the value required. This comparison is facilitated by using a B.D.H. Small Comparator (Fig. 19.2) for which buffer tubes covering the ranges of many indicators

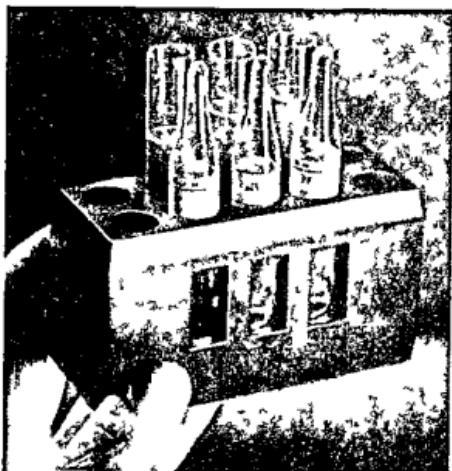


Fig. 19.2 THE B.D.H. SMALL COMPARATOR  
(Courtesy The British Drug Houses Ltd.)

in pH steps of 0.2 can be obtained. To adjust to pH 7.4, bromothymol blue or phenol red is a suitable indicator, and tubes are arranged in the comparator rack as shown in Fig. 19.3.

The tubes must be of hard glass (i.e. free from soluble alkali), perfectly clean and free from scratches

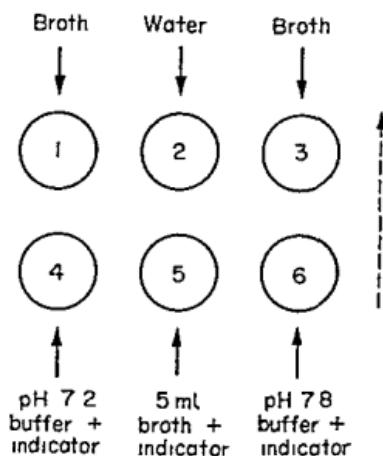


Fig. 19.3 ARRANGEMENT OF TUBES IN COMPAREATOR

Each set of buffers contains a tube of distilled water Only for tube-5 is it necessary to measure the volume accurately

Usually culture media are coloured and this would make accurate colour matching difficult if only the front row of tubes was used With the arrangement shown in Fig 19.3, when the rack is held up to the light and inspected in the direction of the broken arrow, this problem is overcome because the colour in the buffer tubes is corrected by the culture medium tubes in the back row However, if the medium is deeply coloured a pH meter must be used

Over the required pH range, bromothymol blue is more suitable than phenol red because it gives grey shades when mixed with golden brown broth The eye is very sensitive to slight differences in grey and, therefore, matching is easier than with phenol red, which produces shades of orange that are much less easily distinguished

$N/10$  sodium hydroxide solution is added to tube-5 drop by drop from a 2 ml microburette, mixing with a suitably shaped glass stirrer after each addition, until the colour is between the colours of the standards Because the pH falls a little (0.2 to 0.4 units) during sterilisation it is desirable to adjust slightly on the alkaline side of midway From the volume of  $N/10$  sodium hydroxide needed to adjust 5 ml of the medium the volume of  $N/1$  sodium hydroxide required to adjust the remainder is calculated  $N/1$  alkali is used to avoid excessive

dilution of the nutrients, it is added slowly with constant stirring to prevent local high concentrations that might precipitate broth constituents

After the medium has been sterilised its pH should be checked because the *post-sterilisation* pH must lie between 7.2 and 7.8 This may be done with a pH meter, the comparator or the B D H Capillitor (Fig 19.4) The capillitor has the advantage over the comparator that only a drop of broth is necessary Capillary tubes with a single graduation mark are used as pipettes by means of tiny rubber teats Broth is taken up to the mark in one pipette and then injected into a small watch-glass, this is repeated, into the same watch glass but using a fresh capillary, with a special capillary strength indicator solution The two solutions are mixed with the second capillary by drawing up and ejecting several times, and, finally, the mixture is taken up and compared with a set of standard capillary tubes containing bromothymol blue or phenol red buffers In this method the effect of the colour of the broth can be neglected because it has been diluted by an equal volume of indicator solution (cf the comparator) and there is only a very thin layer in the capillary tube

(For further information see B D H (1961))

#### 4 OXIDATION-REDUCTION POTENTIAL

Aerobes can be grown without difficulty in media containing the essential nutrients at the correct pH An occasional complication arises when aerobic culture media are used in screw-capped vials or bottles, if the caps of these are tightly closed during incubation, growth of strict aerobes may not occur They should be at least half full of air, loosely closed and, if profuse growth is required, incubated on their sides to expose a large surface of the medium to the atmosphere This method is easy if flat sided bottles are used because they can be stacked on top of one another in the incubator Care must be taken to prevent the medium from touching the rubber liner of the cap as many rubbers yield extractives that are inhibitory to certain organisms, especially Gram positive (Cooper and Gunn 1957b) species

Suitable conditions for anaerobes can be provided in many ways (Cooper and Gunn, 1957c) but, as liquid media are particularly convenient for sterility testing, the most applicable method is to adjust aerobic fluid media to an appropriate oxidation reduction potential by the inclusion of one or more reducing substances, and to assist the action of these by heating the medium for a short time before use to remove dissolved oxygen.

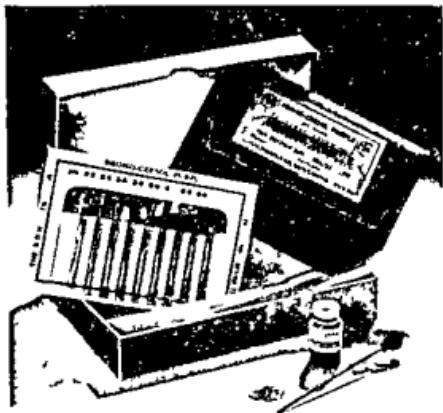


Fig 19.4 THE B D H CAPILLITOR  
(Courtesy The British Drug Houses Ltd)

The most valuable fluid anaerobic media are

- 1 Cooked meat medium (e.g. Robertson's medium)
- 2 Thioglycollate medium (e.g. Brewer's and Linden's media)

The former has a layer of cooked meat, a rich source of natural reducing substances, in the bottom of the container and the latter contains the chemical reducing agent, sodium thioglycollate, the action of which is supplemented by the addition of a little dextrose. Further details are given later.

## 5 STORAGE

Culture media are packed in tubes or bottles. The tubes must be rimless, because rims collect dust and encourage it to drop inside, and they can be closed with cotton wool plugs, metal caps or rubber seals. Bottles are round or flat, in sizes from  $\frac{1}{2}$  to 80 oz, and are closed with a metal screw-cap containing a rubber liner. All containers should be of neutral glass, that will not yield appreciable amounts of alkali to the contents and so alter the pH (see p. 279).

### (a) Cotton-wool Plugs

Non-absorbent cotton wool must be used because the absorbent type becomes saturated during sterilisation and in this state the plugs are inefficient seals, this is partly because they collapse slightly, and partly because motile bacteria can swim through the attached moisture into the tubes. The staple should be long, because short hairs and dust may become detached from low quality wool and fall into the medium, if these come from the outside of the plug, during removal or replacement, they may carry contaminants inside. In addition, foreign particles of this kind can be confused with faint bacterial growth and, also, since cotton wool contains fatty substances promote the growth of micro-organisms (Pollock 1948), they may be responsible for erratic results in critical experiments.

Plugs may be prepared by taking a thin piece of cotton wool, about 4 by 2 inches, turning in loose fibres on the lower edge and then rolling it up. Such a plug should hold firmly together and be inserted or withdrawn easily when held by the incurved little finger, but it must not be too slack in the tube. After fitting, the top should be pulled out to project over and protect the edge of the tube. Coloured wools distinguish the different kinds of media.

### (b) Metal Caps

The most popular type (Oxoid Manual 1961) are made of aluminium to fit  $\frac{1}{2}$ ,  $\frac{2}{3}$ ,  $\frac{3}{4}$ , 1 and  $1\frac{1}{2}$  inch tubes.

They can be used repeatedly, are easily removed or replaced and do not shed particles into the medium. They are available in a large number of colours. Unfortunately, it is rarely possible to buy a batch of tubes of any one size all of which can be fitted with the appropriate caps, some of the tubes are too large while, on others, the caps fit too closely for easy removal and, therefore, may be responsible for semi-anaerobic conditions inside. Further, care is needed to avoid accidental removal of loose-fitting caps, especially when taking a few tubes from a full basket, and, the cap often becomes dangerously hot if several samples are taken from one tube at short intervals and the tube is flamed in between. However, these are small disadvantages compared with the convenience of this type of closure. Instructions for cleaning are provided by the suppliers.

### (c) Rubber Seals

These seals (Test report 1955) have most of the advantages of metal caps and, in addition, when pressed down tightly, they prevent evaporation during storage. To provide optimum conditions for aerobic bacteria they should be loosened slightly.

They fit  $\frac{1}{2}$  in. tubes only, and being of rubber need very careful preparation to remove soluble extractives, some of which are inhibitory to certain micro-organisms.

### (d) Screw-caps with Rubber Liners

Containers closed in this way are hermetically sealed provided the neck of the bottle is unchipped.

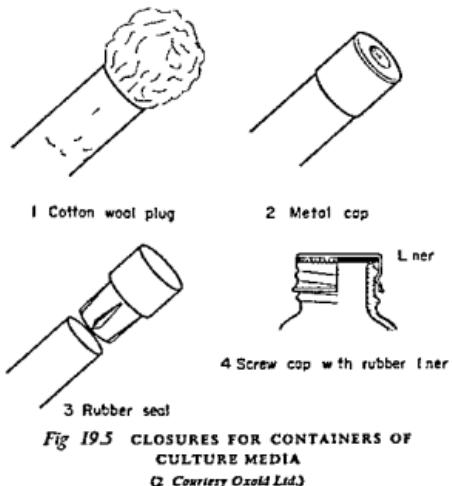


Fig. 19.5 CLOSURES FOR CONTAINERS OF

CULTURE MEDIA

© Courtesy Oxoid Ltd.)

the cap is not distorted and the liner is of the correct thickness. Consequently, media can be stored for long periods without loss of sterility or concentration due to evaporation. The liner must be of a non-inhibitory rubber, silicone is best, but expensive.

Like test-tube caps, screw caps can become too hot to hold, and the rubber liner must never be tightened down on to a hot bottle neck. Strict aerobes will grow well only if the caps are left loose.

Occasionally, if the bottle neck, cap or liner is imperfect, a mould manages to grow up between the

cap and bottle and contaminate the contents. This can be prevented by covering the cap-bottle junction with a plastic ring (see p. 372). The four types of closure are illustrated in Fig. 195.

Culture media should be stored in a dark place (see p. 224). It is also important to reduce loss of water to a minimum otherwise the medium may become too concentrated for optimal bacterial growth; evaporation is prevented by screw caps or rubber seals and can be countered in plugged or capped tubes by storage in a refrigerator.

### Culture Media Used for Sterility Testing

#### 1 BRITISH PHARMACOPEIA REQUIREMENTS

The pharmacopoeia emphasises that media used for sterility testing must be capable of initiating and maintaining vigorous growth of small numbers of aerobic and anaerobic bacteria. Further, it indicates suitable basic ingredients.

#### For Aerobic Organisms

- (a) A meat extract containing a suitable concentration of peptone, or
- (b) The product of enzymic digestion of protein material.

The most frequently used examples of these two classes are Meat Extract broths and Digest broths respectively.

#### For Anaerobic Organisms

Either of the types of aerobic medium containing,

- (a) At least 1 cm depth of heat coagulated muscle at the bottom, or
- (b) A little agar and a substance or substances capable of reducing the oxidation reduction potential sufficiently for anaerobic growth.

These two classes are exemplified by Cooked Meat medium and the Thioglycollate media respectively.

#### 2 METHODS OF PREPARATION

The four examples just mentioned will be used to illustrate the formulae and preparation of typical culture media. In addition, the solid and semi-solid media that are valuable for certain special kinds of sterility test will be described.

#### (a) Meat Extract Broths

A typical formula is—

Lab-Lemco	10 G
Peptone	10 G
Sodium chloride	5 G
Purified water to	1 litre

Lab-Lemco is a meat extract prepared by boiling lean beef in water, filtering and concentrating in

vacuo. Notice the high concentrations of extract and peptone, as explained earlier these are necessary to provide adequate amounts of all the important growth factors.

A satisfactory method of preparation is to weigh the Lab-Lemco on a piece of grease-proof paper and then to roll up the extract in the paper and drop it into a conical flask containing most of the water, previously warmed to 60°C to aid solution. The extract is very sticky and cannot be transferred accurately to the flask with a spatula. The peptone and salt are added and the temperature is maintained on a water bath until, with the assistance of occasional shaking, solution is effected. The pH is adjusted and the solution made up to volume, filtered, packed and sterilised.

A number of commercial, semi automatic filling devices are suitable for distributing accurate volumes of media into tubes and bottles, among these are the Astell and Autopack fillers and the E-mil automatic burettes. If none of these is available an ordinary burette or a Kipps measure may be used, the latter is described in connexion with the pouring of agar plates (p. 474) and, although its accuracy is low, it is particularly useful for distributing sterile liquids, when the volume is not critical, because, unlike many semi automatic devices, it is of a convenient size to sterilise.

For aerobic media either tubes or bottles are suitable. The contents should not occupy more than about half the volume of the container, this is partly to reduce the danger of spillage during handling, partly (when using plugged tubes) to provide a wide gap between the liquid and the plug and so prevent excessive moistening of the latter during autoclaving and partly (when using bottles) to provide plenty of air inside, even if the cap is screwed on tightly.

**Sterilisation** Culture media must be sterilised with great care. Excessive temperatures or times can destroy important growth factors, caramelise sugars, cause precipitation and lead to a drop in

pH Suitable methods will be discussed after sterilisation processes have been considered in detail

(b) *Digest Broth*

A well-known example is Hartley's digest broth. Ox heart or lean beef are used because they are almost free from fat. The small amount present is cut off as completely as possible because it produces turbidity that is difficult to remove by filtration. The meat is minced and added to water in a conical flask which is then warmed to 80°C on a water-bath to soften the tissues. Higher temperatures cause denaturation of the proteins. After cooling to 45°C a pancreatic extract containing the proteolytic enzyme trypsin is added, a favourable pH for its action is produced with sodium carbonate solution, and chloroform is included to inhibit the growth of bacterial contaminants which would flourish at the favourable temperature at which the flask is then incubated (37°C for 6 hr or 45°C for 3 hr). During the incubation the trypsin degrades the meat proteins to peptones. Afterwards its action is stopped by the addition of hydrochloric acid, which also precipitates undigested proteins and the more complex degradation products. Heating in a steamer for half an hour completes the precipitation and drives off the chloroform. The broth is filtered, adjusted to pH, refrigerated if necessary and then packed and sterilised.

Abundant growth occurs in this medium because it is made from fresh meat. There are several alternative methods of preparation (see Sykes 1956) differing chiefly in the source and amount of meat and the enzyme used. Sometimes papain is preferred to trypsin, and one of the advantages is that its optimum temperature is 60°C, consequently, the addition of chloroform is unnecessary because incubation is carried out at a temperature at which very few contaminants will grow.

(c) *Cooked Meat Medium*

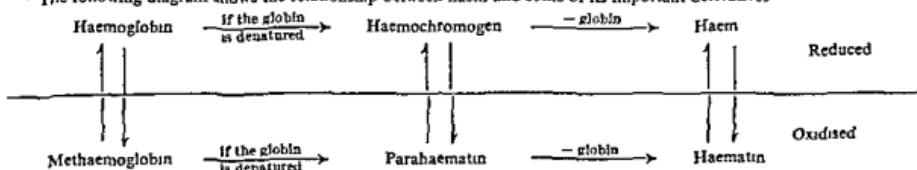
This consists of either of the types of aerobic medium with at least 1 cm of cooked meat at the bottom of

the container. The meat is prepared as follows—

Fat and connective tissue are carefully trimmed from an ox heart. Freedom from fat is extremely important because the meat is in the final medium and turbidity produced by tiny fat globules can easily be confused with bacterial growth. 200 G is minced or, preferably, cut into cubes about  $\frac{1}{4}$ -in square, the latter are cleaner and easier to handle. 200 ml of N/20 sodium hydroxide are added to the meat in a beaker, which is then heated to boiling and simmered for 20 minutes, this procedure emulsifies the remaining fat and neutralises lactic acid from the meat. After cooling, the surface layer of fat is skimmed off and the meat collected by straining through muslin. It is pressed, washed with warm water and spread out to partially dry. Drying makes it easier to put into bottles without soiling their necks. The amount of meat in each container depends to some extent on the depth of broth, it is desirable to aim at 1 cm depth of meat for each 5 cm of medium, and allowance must be made for the settling of the meat to about one half of its original height. Sufficient broth is added to almost fill the container, leaving only the minimum air space necessary to allow for expansion of the contents during sterilisation. It is preferable to use bottles for anaerobic media, but if tubes are chosen the surface should be covered with a half-inch layer of sterile soft paraffin to retard inward diffusion of oxygen. After sterilisation the supernatant broth should be free from turbidity, dehydrated forms of this medium (see p. 233) give no trouble in this respect and can be strongly recommended.

The meat contains a number of natural reducing substances, including unsaturated fatty acids, that absorb oxygen from the medium under the influence of haematin as a catalyst. In addition, an important oxidation-reduction system (parahaematin  $\rightleftharpoons$  haemochromogen) diffuses into the medium. Parahaematin is the oxidised form and is brown in colour, haemochromogen, the reduced form, is pink\*. Consequently, this system acts as an indicator of the state

\* The following diagram shows the relationship between haem and some of its important derivatives



The term haemochromogen is also used for haem derivatives in which the denatured protein is replaced by another nitrogenous compound, the cytochromes, the important respiratory pigments are examples.

of reduction of the medium and if the oxidation-reduction potential is low enough for anaerobic growth the meat, or at least its lower regions, and the adjacent medium are bright pink (Hewitt, 1950) For further information see Sykes (1956)

#### (d) Thioglycollate Medium

The reducing agent used in this medium is sodium thioglycollate and, because this is hygroscopic and not very stable, it is usually prepared by adding the equivalent amount of thioglycolic acid to the broth and neutralising it with sodium hydroxide during the pH adjustment. The stability of thioglycollate solutions with special reference to their use in culture media has been investigated by Cook and Steel (1959a, b)

First, 0.1 per cent of agar is dissolved in a suitable nutrient broth by heating in a steamer. The agar increases the viscosity and, therefore, decreases diffusion of oxygen into the medium. When solution is complete, thioglycolic acid, equivalent to 0.1 per cent of the sodium salt, and 1 per cent of dextrose are added. The dextrose is a valuable extra source of carbohydrate and, since it is a reducing agent, it also decreases the rate of oxidation of the thioglycollate. Usually, 1 part in 500 000 of methylene blue or 1 part in a million of resazurin is included to indicate the state of oxidation or reduction of the medium. Since there is evidence that the above concentration of methylene blue is bacteriostatic to some organisms (Pittman 1946) resazurin is the more satisfactory indicator to use. As oxygen diffuses into the medium and spreads down from the top, the oxidation-reduction potential increases and the indicators show corresponding changes in colour, methylene blue from colourless to blue and resazurin from colourless to pink. Provided that not more than one-fifth of the medium is coloured the conditions at the bottom are suitable for the growth of anaerobic organisms. After the pH has been adjusted the medium is packed in containers that can be hermetically sealed, i.e. screw-capped bottles with rubber liners. The caps are left open during sterilisation to allow the escape of air and hydrogen sulphide. The latter is produced from slight decomposition of the thioglycollate and may darken the medium if not allowed to escape. Immediately after removal from the steriliser the bottles are tightly closed, to prevent resolution of oxygen, and, because the thioglycollate is more stable at low temperatures, stored in a refrigerator until required for use.

Cooked meat medium quickly becomes oxygenated on standing and, unless it has been sterilised

recently, should be heated for about 20 minutes in boiling water before use to restore reduced conditions. Thioglycollate, because of its agar content, absorbs oxygen more slowly and only requires heating if more than the upper fifth is coloured. Cooked meat medium is more sensitive if it has a paraffin seal, there is no need to seal thioglycollate. Cooked meat medium, unless made from dehydrated material, is often slightly turbid and, consequently slight bacterial growth is hard to distinguish; thioglycollate is usually quite clear, although some trouble has been experienced with a turbidity caused by some grades of agar.

For certain sterility tests, cooked meat is added to thioglycollate broths, and the resulting media are extremely sensitive. Plain cooked meat medium is valuable for storing cultures of organisms because it prolongs their life.

#### (e) Solid and Semi solid Media

Solid media are used extensively in pharmaceutical microbiology and a semi solid medium is often used in sterility testing.

Heat is used to dissolve agar, a complex poly saccharide from marine algae, in a suitable fluid medium. On cooling, solid or semi-solid gels are produced according to the amount of agar used. Although agar has no nutritional value, some varieties contain traces of substances that stimulate or inhibit bacterial growth. Only best grades, in which these impurities have been reduced to a minimum by methods that ensure a standard product, should be used. Several commercial grades of New Zealand agar are satisfactory, these are available in powder form, and a concentration of 1 to 2 per cent, depending on the grade, is needed for solid media. It is stirred into warm broth and heated in a steamer or an autoclave until dissolved. The pH is checked while the medium is hot, and readjustment made if necessary.

The hot medium is then clarified through paper pulp. This is conveniently done in a heated stain less-steel funnel (one of 10 in. diameter is a suitable size) connected to a Buchner flask in which a gentle vacuum is produced. A supporting bed of pebbles is put at the bottom of the funnel and covered with soaked, finely shredded paper pulp to a depth of about 3 in. The pulp must be packed down firmly, particularly at the edges. The funnel is heated with an electric mantle or a copper water jacket and the flask is kept warm in hot water to stop the agar solidifying. The first part of the filtrate, which is diluted with water from the pulp, is rejected. The product, known as Nutrient Agar, is packed, usually

in 15 ml quantities, in screw-capped bottles and then sterilised.

Media solidified with agar are more useful than broths for separating and identifying bacteria (Cooper and Gunn, 1957*d, e*), also, they are invaluable for counting the number of living organisms in a suspension and for preparing bacterial vaccines (Cooper and Gunn 1957*f, g*) Agar gels remain solid at 37°C.

*Semi-solid ('Sloppy') Agar* This may be prepared as above, using 0.2 to 0.5 per cent of agar, according to quality. Suitable volumes (often 50 ml) are usually packed in 4-oz screw-capped jars.

The viscosity produced by the agar prevents, to a considerable extent, the absorption of oxygen, and conditions at the bottom of the jar are suitable for anaerobes. It has been used for sterility tests on surgical dressings and ointments.

#### (f) Special Media

A very large number of media are used for special purposes by clinical and industrial microbiologists. These can be classified as follows:

- (i) *Enriched Media* Some pathogens will grow only in media enriched with animal proteins such as blood or serum. An example is blood agar, used in the sterility test for B.C.G. Vaccine of the *British Pharmacopœia*.
- (ii) *Selective Media* These contain substances that, by inhibiting certain organisms and encouraging others, allow selective growth of particular bacteria from a mixture.
- (iii) *Diagnostic Media* By the addition of pH indicators and other chemicals that are affected by the metabolism of a group or species of bacteria it is possible, often, to distinguish different groups or species by the colour developed in or around the colonies, or by a precipitate or gas bubbles produced in the medium.

It would be out of place in this book to discuss the many media in (ii) and (iii) above, and further details should be obtained from textbooks of bacteriology (Mackie and McCartney, 1960, Conn, 1957) and manuals of culture media (Difco Manual, 1953, Oxoid Manual, 1961). The one that has been selected for description (McConkey's Bile Salt medium) is chosen because it could be used to detect *Escherichia coli* in the *British Pharmacopœia* test for the absence of this organism in Smallpox Vaccine, it is selective and diagnostic.

*Blood Agar.* This is prepared by adding 5 to 10 per cent of sterile, oxalated horse blood or citrated human blood to nutrient agar. Nutrient agar melts

at about 95°C but does not solidify again until it has cooled to about 40°C. Consequently, blood can be mixed with it at a temperature (55°C is suitable) that will not coagulate plasma proteins, and bacteria can be incorporated at a temperature (usually 45°C) that will not affect their viability. Blood agar can be used solely as an enrichment medium or as a diagnostic medium to detect and distinguish organisms that can haemolyse blood (Cooper and Gunn, 1957*h*).

*McConkey's bile salt agar* This is a solution of 2 per cent peptone and 0.5 per cent sodium chloride containing 0.5 per cent bile salts and 1 per cent lactose. An indicator, often 0.005 per cent neutral red, is added and the medium is solidified with agar.

Its preparation follows the principles indicated previously except that the lactose must be heated as little as possible because sugars are harmed by overheating, it can be incorporated just before sterilisation or, ideally, a lactose solution of suitable strength may be sterilised by filtration and the appropriate volume added to the melted medium just before use.

The bile salts inhibit some of the intestinal cocci and stimulate the coli-typhoid-dysentery group of bacteria, therefore, the latter are easier to isolate from a sample of faeces. The intestinal commensals (e.g. *Escherichia coli*) in the coli-typhoid-dysentery group ferment lactose, and the resulting lactic acid alters the colour of the indicator (with neutral red the colonies become pink). The pathogens (e.g. *Salmonella typhi*) are usually non-fermenters and their colonies remain colourless.

#### Dehydrated Culture Media

Certain firms supply many culture media in dehydrated condition, as tablets and/or granules, the former are useful for single tubes or small quantities of media. These forms only require solution in purified water and sterilisation. If made according to the maker's directions they produce media of the correct strength and pH.

These products are convenient and time-saving, and, if sufficient tablets or granules of the same production batch are purchased, provide an excellent means of ensuring uniformity of medium throughout a series of experiments. The *United States Pharmacopœia* specifically approves the use of dehydrated media for its sterility tests.

#### Sensitivity of Media

Before a medium is selected for sterility testing it must be shown capable of initiating and maintaining the growth of a wide range of bacterial types even

when only small numbers are inoculated (see pp 224, 448)

A suitable range of bacteria includes common saprophytes (e.g. *Bacillus*, *Micrococcus* and *Pseudomonas* species), pyogenic cocci (e.g. *Staphylococcus aureus* and *Streptococcus pyogenes*) and spore-bearing bacteria pathogenic to man (e.g. *Clostridium* species, especially *Clostridium tetani* and *Clostridium oedematiens*; these are anaerobes and would be used for testing anaerobic media) Some species would be

selected because of their exacting growth requirements

The capacity to initiate the growth of small numbers of bacteria can be investigated by adding to a series of tubes of the medium tenfold dilutions of young cultures. Usually 1 in  $10^5$  to 1 in  $10^8$  dilutions of an 18 to 24 hr culture are suitable. The greater the dilution in which growth occurs the more sensitive the medium. A large number of replicates is always essential

## MOULDS AND YEASTS

Unlike the *United States Pharmacopoeia*, the *British Pharmacopoeia* does not include tests for the absence of moulds and yeasts but, since certain injections are very likely to be contaminated with these organisms, tests are sometimes applied by manufacturers. In designing these, the following points must be considered

### Factors Affecting Growth

#### 1 NUTRIENTS

Moulds and yeasts require the same classes of nutrients as bacteria but the carbohydrate and nitrogen sources are particularly important.

A supplementary source of carbohydrate must be added to most media because a high concentration is essential. Examples are 2 per cent dextrose, 3 per cent sucrose or 4 per cent maltose.

Extracts are used as nitrogen sources but, unlike those chosen for bacteria, are often obtained from vegetable materials, e.g. malt and potato extracts. However, the pathogenic fungi grow better in media containing extracts from animal sources and, therefore, Sabouraud's medium, used in the mould sterility tests of the *United States Pharmacopoeia*, contains a pancreatic digest of casein and a peptic digest of fresh meat. Peptones are often used to supplement or replace extracts and special mycological grades have been developed that give exceptionally rapid and luxuriant growth of moulds and yeasts.

Some moulds, like certain bacteria, grow profusely in stored distilled water.

#### 2 TEMPERATURE

The optimum temperatures for the growth of most moulds and yeasts lie between 20° and 30°C and 25°C is a suitable incubation temperature. The *United States Pharmacopoeia* recommends 22 to 25°C. As many moulds grow rather slowly, tests should be incubated for at least 10 days.

Cultures may be freeze-dried, but investigations of their viability in this condition are less complete than those for bacteria.

Exposure to dry heat at 110°C for 1½ hours is reported to kill all mould spores. They are rapidly destroyed by moist heat, boiling for one minute being sufficient in many cases. This explains why the *United States Pharmacopoeia* does not require mould and yeast sterility tests for liquids and suspensions that have been heated for at least ¼ hour at 100°C.

#### 3 pH

Moulds and yeasts prefer a pH well on the acid side of neutrality. This is confirmed by the flourishing growths that appear in unpreserved Insulin (pH 3 to 3.5) and Adrenaline (pH 3.2 to 3.6) injections. Test media are usually adjusted to between 5 and 6. A pH of less than 5 is avoided when the medium contains agar because this is hydrolysed, with consequent loss of gel strength, if autoclaved at low pH. If a higher acidity is essential it is obtained by adding sterile acid after sterilisation.

#### 4 LIGHT

Most moulds and yeasts seem to grow equally well in the light and dark. Because incubators are not made with windows it is more convenient to incubate in the dark and this also has the advantage that a 'standard darkness' is more easily produced than a 'standard degree of illumination'.

#### 5 AIR

The common saprophytic moulds are very aerobic and to obtain the rapid growth necessary in sterility testing it is preferable to increase the surface exposed to the air by incubating tubes and bottles on their sides.

Yeasts can grow both aerobically and anaerobically, and anaerobic growth deep in winchesters of

unpreserved syrups or syrupy preparations produces alcohol and carbon dioxide and, eventually, the expulsion of the cork by the gas. Special anaerobic media are unnecessary.

## 6 OSMOTIC PRESSURE

Moulds and yeasts are more tolerant of high osmotic pressure than bacteria and are often found as contaminants of unpreserved syrups, semi solid creams and ointments. Additional sodium chloride is unnecessary in mould media.

## 7 SUBSTANCES INIMICAL TO GROWTH

Substances used to prevent the growth of moulds and yeasts are known as *fungistats* while substances used to kill them are called *fungicides*. Among the antifungal agents used for pharmaceutical purposes are the esters of parahydroxy benzoic acid (particularly the methyl and propyl esters), mercury compounds (e.g. phenylmercuric nitrate and thiomersal), benzoic acid, alcohols (e.g. chlorbutol and benzyl alcohol) and phenols (e.g. phenol and chlorocresol). These must be neutralised or 'diluted out' in sterility testing, q.v.

## Culture Media

All the following media are very sensitive and, therefore, suitable for sterility testing.

### 1 SABOURAUD'S (FLUID) MEDIUM

#### (a) United States Pharmacopoeia Formula

Dextrose	2 per cent
Pancreatic digest of casein	0.5 per cent
Peptic digest of fresh meat	0.5 per cent, pH 5.7

#### (b) Alternative Formula

Useful for saprophytes	
Maltose or dextrose	4 per cent
Peptone	1 per cent, pH 5.2

### 2 Malt Extract Broth

Malt extract	1.7 per cent
Mycological peptone	0.3 per cent, pH 5.4

### 3 Honey Broth

Honey	6 per cent
Peptic or pancreatic digest of casein	1 per cent, pH 5.6

(For further information on the constituents and preparation of culture media see the Special report of the Society for General Microbiology (1956) and Conn (1957).)

## REFERENCES

- 1 ANNOTATION (1959) Psychrophilic bacteria *Lancet*, 2, 957
- 2 B.D.H. (1961) *pH values* 7th Ed The British Drug Houses Ltd., Poole
- 3 BISSETT, K.A. (1950) The significance of the bacterial spore *Nature*, 166, 431-432
- 4 BISSETT, K.A. (1955) *The Cytology and Life History of Bacteria* 2nd Ed Livingstone Edinburgh and London
- 5 BISSETT, K.A. (1963) *Bacteria* 3rd Ed Livingstone Edinburgh and London
- 6 BRITISH PHARMACOPOEIA (1963) 10th Ed The Pharmaceutical Press London 1148-1149
- 7 CONN, H.J. (Ed) (1957) *Manual of Microbiological Methods* McGraw-Hill New York, 37-72
- 8 COOK, A.M. and STEEL, K.J. (1959a) The stability of thioglycollate solutions *J Pharm Pharmacol* 11, 216-223
- 9 COOK, A.M. and STEEL, K.J. (1959b) The stability of thioglycollate solutions *ibid* 434-441
- 10 COOPER, J.W. and GUNN, C. (1957a) *Tutorial Pharmacy* 5th Ed Pitman London 510-512
- 11 COOPER, J.W. and GUNN, C. (1957b) *ibid* 524
- 12 COOPER, J.W. and GUNN, C. (1957c) *ibid* 512-514
- 13 COOPER, J.W. and GUNN, C. (1957d) *ibid* 520-522
- 14 COOPER, J.W. and GUNN, C. (1957e) *ibid* 525-526
- 15 COOPER, J.W. and GUNN, C. (1957f) *ibid* 538-539
- 16 COOPER, J.W. and GUNN, C. (1957g) *ibid* 560-561
- 17 COOPER, J.W. and GUNN, C. (1957h) *ibid* 526
- 18 CURRAN, H.R. (1952) Symposium on the biology of bacterial spores Part V Resistance in bacterial spores *Bact Rev* 16, 111-117
- 19 DIFCO MANUAL (1953) *Difco Manual of Dehydrated Culture Media and Reagents* 9th Ed Difco Laboratories Detroit 1, Michigan
- 20 HEWITT, L.F. (1950) *Oxidation Reduction Potentials in Bacteriology and Biochemistry* 6th Ed Livingstone Edinburgh and London 49
- 21 HINSHELWOOD, C.N. (1946) *The Chemical Kinetics of the Bacterial Cell* The Clarendon Press Oxford 27-73
- 22 MACKIE, T.J. and MCCARTNEY, J.E. (1960) *Handbook of Practical Bacteriology* 10th Ed Livingstone Edinburgh and London
- 23 MCBEES, R.H., LAMANNA, C. and WEEKS, O.B. (1955) Definitions of bacterial oxygen relations *Bact Rev* 19, 45-47

- 24 OGINSKY, E. L and UMBREIT, W W (1955) *An Introduction to Bacterial Physiology* W H Freeman and Co San Francisco 55-65
- 25 OXOID MANUAL (1961) *Oxoid Manual of Culture Media* Oxo Ltd. London SE 1
- 26 PITTMAN, M. (1946) A study of fluid thioglycolate medium for the sterility test *J Bact* 51, 19-32
- 27 POLLACK, M R (1948) Unsaturated fatty acids in cotton wool plugs *Nature* 161, 853
- 28 PORTER, J R (1946) *Bacterial Chemistry and Physiology* Chapman Hall London. 102-140
- 29 POWELL, J F and STRANGE, R. E. (1956) Biochemical changes occurring during sporulation in *Bacillus* species *Biochem J* 63, 661-668
- 30 SALLE, A. J (1961) *Fundamental Principles of Bacteriology* 5th Ed McGraw-Hill New York. 82-87
- 31 SPECIAL REPORT OF THE SOCIETY FOR GENERAL MICROBIOLOGY (1956) *Constituents of Bacteriological Culture Media* Cambridge University Press
- 32 SUGIYAMA, H (1951) Studies of factors affecting the heat resistance of spores of *Clostridium botulinum* *J Bact* 62, 81-96
- 33 SYKES, G (1956) The technique of sterility testing *J Pharm Pharmacol* 8, 573-588
- 34 TEST REPORT (1955) Laboratory equipment test report *Laboratory Practice* 4, 185-187
- 35 UNITED STATES PHARMACOPOEIA (1960) 16th Revn Mack Publishing Co Easton Pa 855-859
- 36 WERKMAN, C H and WILSON, P W (1951) *Bacterial Physiology* Academic Press Inc New York. 101-125
- 37 WILLIAMS, O B et al (1952) Symposium on bacterial spores *Bact Rev* 16, 89-143
- 38 WILSON, G S and MILES A A (1964) *Topley and Wilson's Principles of Bacteriology and Immunity* Vol I 5th Ed. Arnold London 104-116



## The Formulation of Injections

THE formulation of injections involves careful consideration of the following factors

- 1 The route of administration
- 2 The volume of the injection
- 3 The vehicle in which the medicament is to be dissolved or suspended
- 4 The osmotic pressure of the solution
- 5 The need for a preservative
- 6 The units in which the formula is expressed
- 7 The hydrogen ion concentration of the solution
- 8 The stability of the medicament—stabilisers may be necessary
- 9 The specific gravity—when the injection is used for spinal anaesthesia
- 10 For suspensions—presentation in an elegant form from which accurate dosage is possible

### ROUTE OF ADMINISTRATION

The administration of drugs other than by the alimentary tract is known as parenteral treatment and the fluid preparations used are called injections. The most important of these are

#### 1 Intracutaneous or Intradermal Injections

These are made into the skin, between the inner layer, or dermis, and the outer layer, or epidermis (Fig 20 1a). The skin of the front of the left forearm is usually selected. The volume that can be injected intradermally is necessarily small, usually 0.1 to 0.2 ml. The route is used mainly for diagnostic purposes in investigations of immunity and allergy.

#### 2 Subcutaneous or Hypodermic Injections

These are made under the skin, into the subcutaneous tissue (Fig 20 1a). The volume injected is 1 ml or less, usually into the upper arm. This is the most popular route because it is convenient for patient and doctor.

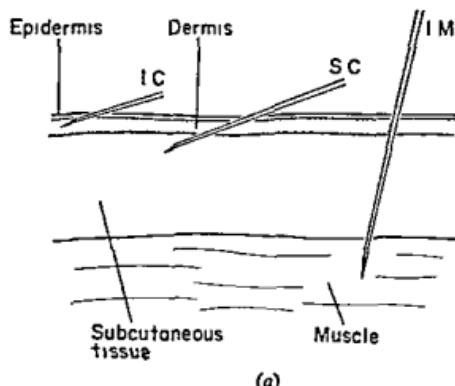
#### 3 Intramuscular Injections

These are made into a muscle, the needle passing through the skin, subcutaneous tissue and the membrane enclosing the muscle, and opening into muscle tissue (Fig 20 1a). The muscles of the shoulder, thigh or buttock are usually selected. The

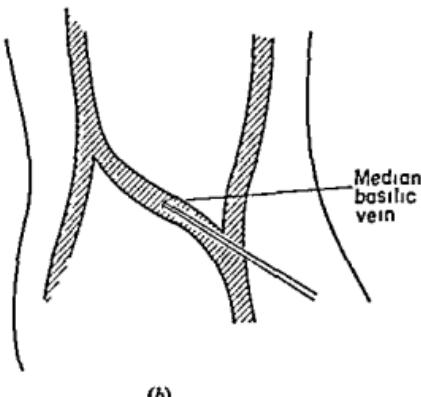
volume rarely exceeds 2 ml. Aqueous or oily suspensions and oily solutions cannot be given subcutaneously because they cause pain and irritation, or intravenously, because blockage of small blood vessels might occur, muscle tolerates them relatively well and, therefore, the intramuscular route is used for their administration.

#### 4 Intravenous Injections

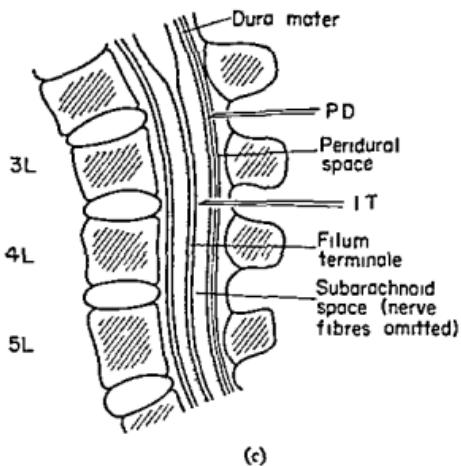
These are made into a vein and, therefore, are introduced directly into the blood stream. The median basilic vein, near the anterior surface of the elbow, is usually selected because it is close to the surface, easily located and connects with the other large veins of the arm (Fig 20 1b). The volume injected varies from 1 ml or less, to 500 ml or even more. Small volume injections are given by this route when a very rapid effect is required, as in the administration of certain anaesthetics (e.g. thiopentone). Large volume injections, often called perfusion or, more correctly, infusion fluids, are frequently needed to replace body fluids lost from the circulation as a result of shock, severe burns, vomiting, diarrhoea, haemorrhage and certain diseases; direct injection into the blood stream is the best way of ensuring that these large volumes are quickly dispersed throughout the body.



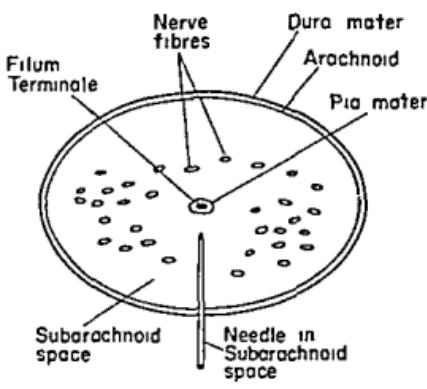
(a)



(b)



(c)



(d)

Fig 20.3 ROUTES FOR INJECTION

### 5. Intrathecal Injections

These are made into the subarachnoid space that surrounds the spinal cord. The latter is enclosed in three coats, a tough outer one, known as the dura mater, a middle one called the arachnoid, and the inner pia mater. Between the arachnoid and pia mater there is a space called the subarachnoid space, containing a fluid known as the cerebrospinal fluid, which, among other functions, serves as a liquid cushion for the spinal cord, reducing the effects of sudden jolts. The subarachnoid space is relatively narrow for most of its length, and in this part it

would require great skill to make an injection without entering the spinal cord and causing serious damage. The cord terminates at about the first lumbar vertebra, passing into a narrow column called the filum terminale, from the beginning of which the subarachnoid space widens proportionally (Fig 20.1 c, d). Intrathecal injections are therefore made into the subarachnoid space well below the termination of the spinal cord, usually between the third and fourth (Fig. 20.1c) or fourth and fifth lumbar vertebrae. At this site the filum and nerve fibres lie in a considerable volume of cerebrospinal fluid

which protects them from accidental damage from the needle.

The patient's back is flexed, thereby providing a passage for the needle between the vertebrae. The volume injected is usually less than 10 ml and the route is used for spinal anaesthetics (e.g. amethocaine hydrochloride) and antibiotics (e.g. streptomycin sulphate in the treatment of tubercular meningitis). Cerebrospinal fluid, when required for diagnostic purposes, is withdrawn in a similar manner.

### 6 Intracisternal Injections

The needle is inserted in the mid line between the atlas and axis (first and second cervical vertebrae respectively) and directed forward and upwards. It passes through the occipito-atlanto-occipital ligament and enters the cistern (Fig. 20.2). If it is inserted too far it pierces the medulla, with disastrous results.

The intracisternal route is used principally to withdraw cerebrospinal fluid (CSF) for diagnostic purposes, e.g. when a lumbar puncture (i.e. withdrawal of CSF by the intrathecal route) has been unsuccessful, or when it is desired to measure the difference in pressure between the CSF in the cistern and the lumbar subarachnoid space. Occasionally antibiotics are administered by this route.

### 7 Peridural Injections

The peridural space is seen in Fig. 20.1c between the dura mater and the inner aspect of the vertebrae.

## VOLUME OF THE INJECTION

Usually the volume of an injection depends on the solubility of the medicament but it may also be influenced by the prescriber's preference for a particular route.

Intracutaneous injections must be of very small volume because they do not disperse quickly from their relatively non-vascular site and even a fraction of a millilitre remains as a small blister for some time after injection.

Only the intravenous route is really suitable for very large volumes, the injection being allowed to drip slowly into a vein from a bottle suspended over the patient's bed—a procedure known as infusion.

Very occasionally, large volumes are given by infusion into muscle or subcutaneous tissue but the injection is not quickly carried away, as it is when the intravenous route is used, and, consequently, it tends to collect and cause a swelling. If it is essential to infuse by either of these routes a small amount of

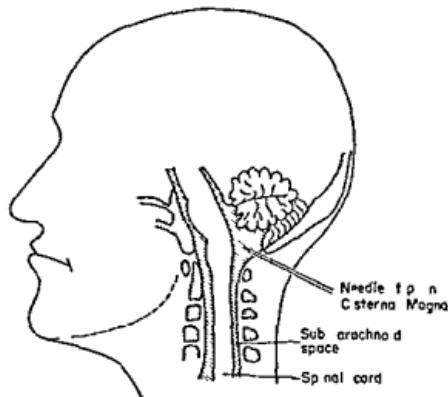


Fig. 20.2 ROUTE FOR INTRACISTERNAL INJECTION

Therefore, it is that portion of the vertebral canal not occupied by the dura mater and its contents. The space extends along the full length of the spinal column. Injections are made into its posterior aspect in different regions, e.g. thoracic, lumbar and sacral. The peridural route is sometimes used for spinal anaesthetics because it has several rather specialised advantages over the intrathecal route for certain operations (see Ciba, 1955). Considerable skill is required to place the injection inside the very narrow peridural space.

the enzyme hyaluronidase is sometimes given with the injection, thus hydrolyses hyaluronic acid, a viscous component of the 'cement' that holds tissue cells together and, therefore, the fluid is able to diffuse between the cells and away from the site quickly. The hydrolysis is reversible and the acid is reformed about 20 minutes after completion of the injection.

The volume also influences other aspects of formulation. Large volumes must be made isotonic. Intravenous injections of dose greater than 15 ml may not contain a bactericide and may not be sterilised by heating with a bactericide.

The volume must be convenient to administer. More than 20 ml is unsuitable for injection by a syringe and it is hardly worth setting up an infusion unit for less than 250 ml. Often the volume can be reduced by dispensing a hypertonic solution and directing slow intravenous injection (see p. 248).

## THE VEHICLE

There are four main types of sterile preparation intended for injection

- 1 Solutions of medicaments
- 2 Dry solids intended to be dissolved in a suitable vehicle before use Hypodermic solution tablets are a special example of this class
- 3 Suspensions of medicaments
- 4 Dry solids intended to be suspended in a suitable vehicle before use

Recently, a new type of injection has been introduced—the sterile emulsion. It is used to make safe the injection of oily substances by the intravenous route To avoid thrombosis and other reactions, the oil must be in very fine globules, and this is achieved by emulsification. An example is Phyto-menadiene (Vitamin K<sub>1</sub>) Injection in which the non-toxic emulsifying agent lecithin is used, most of the globules are less than 3 microns in diameter and, therefore, approximate to the size of the fat chylomicrons normally found in the blood stream

Water is used as the vehicle for most injections because aqueous preparations are tolerated well by the body and are the safest and easiest to administer An oily vehicle may be necessary (a) if the medicament is insoluble or only slightly soluble in water, (b) when a depot effect is desired, and (c) when an oily medium is more suitable for a diagnostic procedure For example, (a) dimercaprol has poor solubility in water, (b) oestradiol benzoate, progesterone, testosterone propionate and deoxycortone acetate are insoluble, or almost insoluble, and are required in depot form to continuously replace deficient secretions, (c) propylthiouracil is often preferred in oily suspension for use as a contrast medium for X ray examination of the respiratory tract because this is less irritating than the aqueous suspension

Oily injections suffer from the following disadvantages (a) they may be too viscous in cold weather for administration without warming, (b) they often cause pain on injection, (c) they make the syringe and needle difficult to clean, and (d) they must be injected with great care to avoid accidental intravenous injection which could lead to thrombosis For the last reason the *British Pharmacopœia* directs that oily injections must be labelled 'For intramuscular use only', this does not apply to certain contrast media—Fluid and Viscous Iodised Oil Injections and Propylthiouracil Oily Injection, because these are not injected into tissues but are introduced into the internal cavities that are under investigation

(e.g. the lungs) by means of a soft rubber tube passed along the normal tract

Very occasionally, alcohol is used to dissolve the medicament and maintain its stability, but the solution must be diluted with an aqueous vehicle just before administration, to prevent pain and tissue damage

## AQUEOUS VEHICLES

### 1 WATER FOR INJECTION

#### *(a) Pyrogens*

The injection of distilled water may cause a rise in body temperature, water producing this reaction is said to be pyrogenic (meaning 'producing fever') and water free from this effect is described as a pyrogenic

At the beginning of this century fever was a frequent complication of intravenous injections After several workers (see Whittet, 1954) had shown the cause to be microbial contamination of the water used as a solvent, Seibert, in 1923, identified the pyrogenic agent as a filterable, thermostable and non-volatile substance of bacterial origin

There appear to be several different pyrogens with closely related chemical structures They are produced chiefly by Gram-negative bacteria and may form part of the endotoxin (the most important O somatic antigen) of these organisms (Todd, 1955) In smooth forms the endotoxin consists of a complex of a pyrogenic lipopolysaccharide, a protein and an inert lipid. The lipid part of the lipopolysaccharide is the main pyrogenic agent but combination with the polysaccharide increases its activity, probably by making it more soluble In rough bacteria the active lipid is associated with the protein instead of the polysaccharide and in this form is less active Some lipopolysaccharides have been highly purified by Westphal and others, and found to have a molecular weight of about one million, i.e. they are about the size of a very small virus Unlike the complete endotoxin, the lipopolysaccharide fraction is water-soluble and, consequently, is found in the medium in which the organism is grown.

The pyrogens in intravenous fluids must be extremely active because relatively few contaminating bacteria are required to produce a pyrogenic solution.

(For further information on endotoxins and antigens, see the sections on bacterial variation and immunity in Cooper and Gunn (1957))

The presence of pyrogens in an injection is most serious when the volume is large There are three reasons for this (a) a large-volume injection will

contain a correspondingly large amount of pyrogen, (b) large volume injections (infusion fluids) are usually given intravenously and, consequently, the pyrogen will have a more rapid effect, (c) patients receiving infusion fluids are often dangerously ill and the effect of the rise in temperature could be disastrous (e.g. see Dykes, 1962) Therefore, it is of the utmost importance to eliminate pyrogens from injections, especially those given in large volumes intravenously.

The sources of pyrogens in injections may be the solvent, the medicament, the apparatus and the method of storage between preparation and sterilisation. Possibly the most important is the solvent.

Distilled water for injections is prepared from potable water and since several tap waters have been shown to be pyrogenic the first aim in the preparation of Water for Injection must be to remove or destroy pyrogens. This is complicated because pyrogens are:

- (i) Thermostable—to destroy some types, temperatures much higher than those used in the official sterilisation processes are necessary
- (ii) Water-soluble—therefore, they are not removed by the usual types of bacteria proof filter
- (iii) Unaffected by the common bactericides

Consequently none of the methods of sterilisation of the *British Pharmacopoeia* can be relied upon to eliminate pyrogens.

However, there are also

- (iv) Non-volatile, and this provides a way of removing them from water Ordinary distillation is not satisfactory, however, because a small amount of pyrogen is carried over into the receiver, dissolved in the spray that is entrained in the steam. But, if a trap is fitted to the distilling flask, to stop this entrainment, an apyrogenic distillate can be obtained. The trap contains a series of baffles on which the spray is removed by impingement, an example is shown in Fig 20.3. Because, in the past, so much emphasis has been placed on the importance of entrainment as a cause of pyrogenic contamination of distilled water it is interesting to read the work of Shotton and Habeeb (1954), which suggests that the danger of entraining pyrogens may have been over estimated.

The ability of certain bacteria to multiply in distilled water was mentioned in the section on Factors affecting the growth of bacteria in chapter 19. The number of bacteria present depends on the post-distillation storage conditions. Inadequate protection from air and storage at a temperature that favours bacterial growth causes rapid increase in the bacterial content, e.g. Martindale found

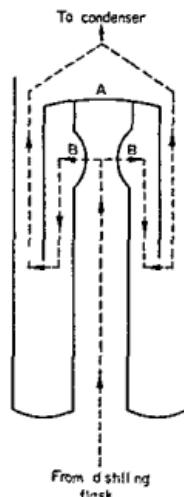


Fig. 20.3 STILL HEAD  
A = glass hood. B = holes in tube.  
Dotted line shows interrupted path of steam.

3,800,000/ml after 15 days. Distilled water has become pyrogenic after 4 hours in an unprotected container at room temperature. Consequently, distilled water for parenteral solutions must be sterilised immediately after collection from the still. The only exception is when the water is used at once for making an injection that requires sterilisation, then, provided the injection is sterilised immediately after preparation, freshly distilled, non-sterilised water may be used.

Because of the great danger from pyrogens in fluids given by infusion, the *British Pharmacopoeia* gives a special warning of the need for immediate sterilisation, in the monographs of the injections used in this way, e.g. Dextrose, Sodium Chloride, Sodium Chloride and Dextrose, Sodium Lactate and Compound Sodium Lactate Injections.

The interpretation of 'immediate sterilisation' may seem difficult, particularly where very large batches of infusion fluids have to be processed. Ideally, quantities that are too big to be sterilised immediately should not be prepared, but if large batches are unavoidable it is important to remember that the amount of pyrogen produced during storage depends on the number of bacteria originally present and their rate of multiplication. Therefore, if the distilled water, or the injection, is badly protected during preparation, if indifferently cleaned receivers and

containers are used, and if the latter are inadequately closed and stored at room temperature, contamination will be high and multiplication considerable. On the other hand, if care is taken to reduce the number of contaminating bacteria by protection during preparation, by using well-cleaned containers and by storage at a temperature unfavourable to bacterial growth (e.g. in a refrigerator) the amount of pyrogen produced will be negligible. In these circumstances unsterilised injections can safely be left overnight.

#### (b) Preparation of Water for Injection

Metal stills are suitable for preparing Water for Injection if they are well baffled and made from a material from which toxic metals such as copper, cannot be leached. Nowadays, special grades of stainless steel are often preferred but, in the past, various metals, heavily coated with tin to prevent extraction, were used. A satisfactory alternative is a still of neutral glass, neutral glass (see 'Containers') is necessary because, unlike ordinary (limesoda) glass, it yields only minute amounts of alkali to the water and is very heat resistant. An advantage of glass stills is ease of cleaning, unless the complete still, including the condenser, is cleaned frequently, dirt and micro-organisms will collect inside and contaminate the distillate.

A useful type of glass still is shown in Fig. 204. Its mode of operation will be described to illustrate the precautions necessary in the preparation of Water for Injection.

The water is heated by a chromium plated immersion heater which can be separated from the distilling flask for cleaning. The glass parts are then treated with a strong oxidising agent, such as chromic acid, to destroy any pyrogen attached to their surfaces. Scale is removed from the heater by rinsing with 5 per cent hydrochloric acid. Cleaning is followed by thorough rinsing with potable and then freshly distilled water.

The cooling water from the condenser is fed continuously to the distilling flask from which excess overflows to waste through a constant level device. By running the water at a suitable speed it can be made to leave the condenser at about 90°C without seriously affecting the efficiency of condensation. At this temperature dissolved gases are largely expelled, and escape from the hole at the top of the constant level device. Any gas taken into the still is driven off during distillation and escapes from the bleed on the outlet from the condenser. Removal of acid and alkaline gases (such as carbon dioxide and ammonia, respectively) ensures that

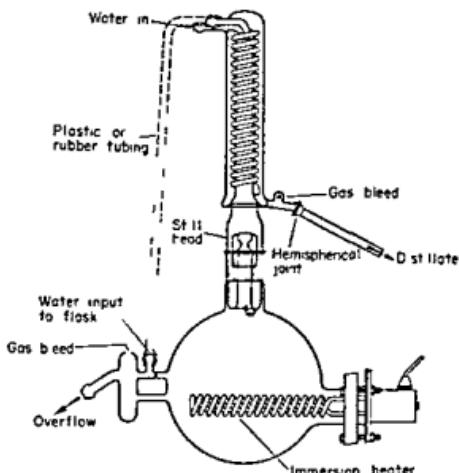


Fig. 204 WATER STILL  
(Courtesy of Loughborough Glass Co Ltd)

the pH is as near to neutral as possible. In this connexion it should be remembered that if the still has been out of use for some hours (e.g. overnight) gases from the atmosphere will have redissolved in the water left in the distilling flask and although most of this will escape from the outlet bleed it is safer to reject the first part of the distillate (i.e. about  $\frac{1}{10}$  of distilling-flask volume). This procedure will also remove dust and micro-organisms that have gained access to the condenser while it was out of use.

The immersion heater boils the water very rapidly and without bumping, and the efficient baffling system in the still head removes entrained droplets from the steam.

If a simple still without a continuous supply of water is used and distillation is continued almost to dryness, certain inorganic salts may hydrolyse to produce volatile substances that will contaminate the distillate ( $\text{e.g. } \text{MgCl}_2 + \text{H}_2\text{O} = 2\text{HCl} (\text{volatile}) + \text{MgO}$ ). Consequently, in this type of still, the last tenth of the original volume in the flask should not be collected.

The distillate is collected in a perfectly clean, pyrogen free container that is protected by a dust-cover during distillation to prevent organisms in the air from dropping inside. When the container is full it is sealed at once and sterilised immediately, or if desired its contents may be distributed into equally clean smaller containers first. Immediate sterilisation prevents pyrogen production by the few

bacteria that may have gained access. If the water is used without delay for making injections the sterilisation may be deferred until the latter are sterilised provided this is done as soon as possible.

The purity of apyrogenic water can be checked by measuring its electrical resistance. This is very high for pure water but is lowered by traces of the ions of mineral salts because they increase the conductivity. Entrainment or a leaking condenser, either of which could make the distillate pyrogenic, will carry mineral salts into the receiver and the resulting decrease in resistance will give warning of probable contamination with pyrogen. The resistance is measured with a conductivity meter. High quality distilled water has a specific resistance of 500,000, or more, ohm/cm and it is inadvisable to use the distillate if there is a fall to about half of this value.

### **Summary**

Clean glass parts of still frequently with an oxidising agent.

Reject first part of distillate

Continuous still, have water entering flask at about 90°C

Non continuous still, reject last part of distillate  
Protect receiver and close immediately when full

**Protect receiver  
Sterilise at once**

(d) *Test for Pyrogens*

The *British Pharmacopæia* includes a test designed to limit the pyrogenicity of Water for Injection. Rabbits are given intravenous injections of the water, under carefully controlled conditions, and the rise in rectal temperature is found. Details are given in Appendix 2.

## 2 WATER FOR INJECTION FREE FROM CARBON DIOXIDE

Some important organic medicaments, e.g. the barbiturates and sulphonamides, are weakly acidic and only slightly soluble in water, consequently, they are used for injection as their much more soluble sodium salts. However, if the Water for Injection used as a solvent contains dissolved carbon dioxide the resulting slight acidity is sufficient to

Clean glass parts of still frequently with an oxidising agent	To destroy pyrogen film
Reject first part of distillate	Contains dissolved gases and dust and micro-organisms from the condenser
Continuous still, have water entering flask at about 90°C	Removes most of dissolved gases
Non continuous still, reject last part of distillate	To avoid decomposition of dissolved salts
Protect receiver and close immediately when full	To keep out as many bacteria as possible
Sterilise at once	To prevent pyrogen production by the few bacteria present

(For further information on the preparation of Water for Injection see Perkins (1956), Saunders and Shotton (1956), and Whittet (1959))

(c) *Purified Water B.P*

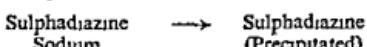
The *British Pharmacopoeia* allows the use of water purified by ion exchange (see Calder, 1958, Cooper and Gunn, 1957a) for all purposes except the preparation of injections. This exception is because there is some doubt about the efficiency of pyrogen removal by demineralisation techniques. However, several workers (see Cook and Saunders (1962) and Whittet, 1956, 1961) have produced apyrogenic water using ion exchange resins and it seems probable that reliable methods will be developed eventually. One of the problems will be to remove or destroy micro-organisms filtered from the water by the resins, these might become a very serious source of pyrogens.

decompose the salts and precipitate the free compounds, e.g.

**Phenobarbitone Sodium + Carbon dioxide**  
**(Soluble 1 part in 3 parts**  
**of water.)**

+ Water = Phenobarbitone (Precipitated)  
 (Soluble 1 part in 1,000 parts  
 of water).

*± Sodium carbonate*



(Soluble 1 part in 2 parts of water)      (Only very slightly soluble in water)

A further example is Aminophylline, this is a mixture of approximately equimolecular proportions

of theophylline and ethylenediamine. Theophylline has replaceable acidic hydrogen atoms and, therefore, combines with amines to give water soluble salts. One part of aminophylline is soluble in 5 parts of water but dissolved carbon dioxide liberates and precipitates the theophylline which is only soluble 1 part in 120 parts of water.

For injections of these substances it is necessary to use Water for Injection from which carbon dioxide has been removed, and this can be done by boiling Water for Injection for at least 10 minutes. Meanwhile, exposure to the air should be minimised by covering the mouth of the flask with a small inverted beaker, a cotton-wool plug is unsuitable because it may shed fibres into the liquid. Water collected directly from a still of the type described above is sufficiently free from carbon dioxide but it must not have been left standing. If possible, carbon-dioxide-free water should be used at once, but storage is sometimes necessary because certain of the injections for which it is the solvent (e.g. Phenobarbitone Injection) must be prepared immediately before administration. In this case, significant reabsorption of carbon dioxide is prevented by immediate but gentle filling into suitable containers which are well-filled and well sealed. Ampoules give the best protection and are usually appropriate because the volumes required are generally small. Immediate sterilisation is essential (see Water for Injection).

(Water for Injection free from Carbon Dioxide is unnecessary for Thiopentone Injection because the official substance is a mixture of thiopentone and sodium carbonate and the latter will keep the salt in solution by displacing the reaction to the left.)

### 3 WATER FOR INJECTION FREE FROM DISSOLVED AIR

The use of the solvent Water for Injection free from Dissolved Air is one of several methods (see p 267) by which sensitive medicaments can be protected from oxidation.

It is made in essentially the same way as Water for Injection free from Carbon Dioxide. Freshly distilled apyrogenic water is boiled for 10 minutes and precautions are taken to exclude air during cooling. It should be used immediately. If a large volume is required it is advisable to both cool the water and prepare the injection under nitrogen.

Since all the injections made from this solvent can be sterilised by heating in an autoclave or (in one case, Promazine Injection) by passage through a bacteria proof filter, and these are methods in which the injection can be prepared completely before issue it is never necessary to pack and store

this solvent (cf. 'Water for Injection free from Carbon Dioxide').

The injections in which it is used are listed in Appendix 5. In every case the residual air in the container must be replaced by an inert gas.

## NON-AQUEOUS VEHICLES

### 1 OILS

Six of the oily injections of the *British Pharmacopœia* (Deoxycortone acetate, Nandrolone phenylpropionate, Oestradiol benzoate, Progesterone, Testosterone phenylpropionate and Testosterone propionate) are simple solutions and for these the prescribed solvents are a suitable fixed oil, a suitable ester (e.g. ethyl oleate) or a mixture of both. Suitable alcohols may be included in the solvents for deoxycortone acetate, oestradiol benzoate and progesterone.

*Fixed oils* for injection must not contain mineral oils or solid paraffins as these would not be metabolised by the body and, therefore, might eventually cause tissue reactions and even tumours. They must be free from rancidity, because rancid oils contain much free fatty acid, and from any material that might cause irritation or side effects. In the *International Pharmacopœia* (1955) tests are described for the absence of mineral oils and paraffins. Rancidity can usually be detected by the odour, taste and a high acid value.

Arachis oil is specified for the B.P. injections of Dimercaprol and Propyl iodone (Oily) but has the disadvantages of thickening slowly on exposure to air and becoming rancid. For the simple solutions, for which no particular oil is directed, the alternatives include sesame (probably the most stable because it contains natural substances that prevent rancidification), cottonseed and maize. In the United States it is recommended that the label should show the name of the oil because sensitivity has been reported in some cases.

Esters give less-viscous preparations that are easier to inject, particularly in cold weather, but a reduction in the length of action of depot preparations has been found when ethyl oleate was used instead of oil. Possibly this is because the maximum prolongation effect will be obtained from a depot if it is spherical and, therefore, absorption will be more rapid from the less viscous ester preparations because of their greater tendency to spread and offer a bigger surface to the tissue fluids.

Permission has been given to use certain alcohols in three of the official injections because, occasionally, the prescribed concentration of medicament is greater than its solubility in fixed oils, ethyl oleate

or mixtures of both Benzyl and ethyl alcohols improve the solvent properties of these vehicles in concentrations that are harmless and non irritant by the intramuscular route, 4 per cent of benzyl alcohol and 10 per cent of a mixture of benzyl and ethyl alcohols have been used. Table 201 of solubilities indicates the advantage of using a solvent containing an alcohol for deoxycortone acetate, progesterone and, to a lesser extent, oestradiol benzoate. On the other hand, the high solubility of testosterone propionate in arachis oil and ethyl oleate makes the use of alcohols unnecessary, and consequently, the pharmacopoeia does not mention them in this case or for the injections of nandrolone and testosterone phenylpropionates to which a similar explanation applies. The numbers in the table are the parts of solvent in which one part of the medicament is soluble.

jection is less than the solubility of dimercaprol in arachis oil

## 2 ALCOHOL

Alcohol is chosen as an injection solvent only when other methods of presentation are impracticable. Its own physiological activity and the pain and tissue damage it causes unless administered by slow intravenous injection are serious disadvantages. The only medicament for which it is used as the main solvent is Hydrocortisone (see B P C monograph). This is almost insoluble in water but because it is given intravenously (to produce a very rapid effect in the crisis of Addison's disease) the use of suspensions or oily preparations would be dangerous. A solution is made in 50 per cent alcohol and sterilised in ampoules, because alcohol is too volatile to be heated in multi dose containers. Just before

Table 201

	Water	Arachis oil	Ethyl oleate	Alcohol 95%
Deoxycortone acetate	Almost insoluble	140	150	50
Oestradiol benzoate	Insoluble	500	200	150
Progesterone	Insoluble	60	60	8
Testosterone propionate	Almost insoluble	35	20	6

The introduction of new materials to other fields of pharmaceutical formulation has provided substances of potential use as non aqueous vehicles for parenteral preparations and among these are polyethylene glycols (Carpenter and Shaffer, 1952), isopropyl myristate (Platcow and Voss, 1954) and polyoxyethylene oleic triglycerides (Labrafils). New materials must be exhaustively tested for freedom from toxicity, and on this ground many fail.

### *Dimercaprol Injection*

This contains an unusual solvent—benzyl benzoate. Dimercaprol is a liquid which is formulated in oil because aqueous solutions are unstable. The original formula was devised in America where a solution of twice the strength of B P injection is used. It was found that although the amount of dimercaprol in this strong preparation would not dissolve in arachis oil it was miscible with benzyl benzoate and the resulting mixture could be diluted with arachis oil to give an injection that was stable, even to dry heat sterilisation, if protected from air. The British Pharmacopoeia uses the same solvent, because of its stabilising effect, although the strength of its in-

use, 20 ml of the alcoholic solution is diluted with 25 times its volume of either Sodium Chloride or Dextrose Injection and administered by infusion. Careful labelling is necessary to prevent accidental injection of the undiluted alcoholic solution.

## 3 PROPYLENE GLYCOL

Digoxin is almost insoluble in water and, in the injection of the 1958 B P, a solution was made in 70 per cent alcohol, this had to be diluted with 10 times its volume of Sodium Chloride Injection and given intravenously, by slow injection to ensure rapid dilution with the blood (cf hydrocortisone). Recent work by Burroughs Wellcome and Co has shown that a stable solution can be produced by using a solvent containing 40 per cent propylene glycol, 10 per cent alcohol and water, and buffering the pH to about 7. Consequently, this has now replaced the old formula. In addition to not requiring dilution before use the new injection has the advantage of being 5 times as strong as the diluted 1958 solution and, therefore, the dose can be given in a much more convenient volume, e.g. 2 ml instead of 10 ml.

and the concentration of the hypotonic solution of sodium chloride which just causes haemolysis is called the fragility point of the red cells. It is usually between 0.42-0.48 per cent of sodium chloride, averaging 0.54 per cent, and may be determined as follows—

Set up 6 small tubes, labelled 1-6, and place in each 2 ml of solution of sodium chloride of the strengths stated in Table 20.2. To each add a few drops of oxalated blood, mix, and set aside. In some tubes the red blood cells will settle to the bottom, leaving a colourless liquid above, and proving that no haemolysis has taken place (N.H. in Table). In some tubes haemolysis will occur, and the haemoglobin from the burst cells will dissolve to form a clear bright liquid, becoming deep red when haemolysis is complete (C.H. below). In the state of partial haemolysis (P.H.) the liquid will be from orange to pale red in colour.

Normal results are shown in Table 20.2

hypotonic solutions, but these are always well above the fragility point, and are therefore sufficiently accurate for practical purposes.

## 2. EFFECTS OF HYPERTONIC SOLUTIONS ON BLOOD-CELLS

When a blood-cell is placed in a hypertonic solution (e.g. a 5 per cent solution of sodium chloride) the consequence is the reverse of that above, in other words, water passes outwards through the cell wall in an attempt to reach equilibrium. The cell therefore shrinks, and the cell wall appears crenate in outline, this change is called crenulation. Blood cells which have undergone crenulation return to normal when pressure becomes equal on both surfaces of the cell wall, consequently a solution which causes crenulation produces only temporary damage—grossly hypertonic solutions may therefore be administered without permanent damage to the blood-cells.

Table 20.2

Tube	1	2	3	4	5	6
Strength of solution of sodium chloride	0.3% C.H.	0.35% C.H.	0.4% C.H.	0.45% P.H.	0.5% N.H.	0.55% N.H.
Effect						

A solution containing 0.45 per cent of sodium chloride has a molecular concentration of about 0.015 per cent (see p. 253). Solutions with a molecular concentration lower than 0.015 per cent may therefore bring about haemolysis, and thus cause irreparable damage. A small volume (e.g. up to 5 ml) of a solution with a molecular concentration lower than 0.015 per cent, injected intravenously, quickly mixes with the blood plasma, and is raised above the fragility point before it can do appreciable damage. A large volume (e.g. 100 ml) of a solution with a molecular concentration lower than 0.015 per cent dilutes the blood plasma to below fragility point for a short time, and considerable haemolysis ensues. It is important, therefore, that solutions with a molecular concentration lower than 0.015 per cent should be rendered isotonic, when a considerable volume is to be injected—even with smaller volumes the adjustment is desirable.

It will be noticed that accuracy in adjustment, although commendable, is not absolutely necessary provided the molecular concentration is raised appreciably above that of the fragility point.

As will be seen, the molecular concentration method of calculation usually produces slightly

In practice, hypertonic solutions are commonly used to simplify the technique of administration. For example, Tryparsamide is given in doses of 2 G dissolved in water to produce 7.5 ml, and the only apparatus needed for injection is a 10 ml syringe and a needle—the period of injection is about five minutes.

Tryparsamide is the sodium salt of a complex phenylarsomic acid, and has a molecular weight of 305. Assuming that the compound yields two ions, the molecular concentration method of calculation (see later) indicates that a 4.5 per cent solution would be isotonic, and consequently it would be necessary to dissolve a dose of 2 G in sufficient water to produce 44 ml in order to produce an isotonic solution. This volume would entail an elaborate technique for the physician, and would be wearisome to the patient. Hence the above mentioned hypertonic solution is preferred.

## SUMMARY

### Hypotonic Solutions

When injected into the blood stream these may cause haemolysis, the conditions most likely to

bring this about being (a) a large volume, (b) an unadjusted solution with a molecular concentration below 0.015%, i.e. below the fragility point of the red blood cells

### Hypertonic Solutions

When injected into the blood stream these may cause crenulation of cells, but these return to normal with equalisation of the osmotic pressure. Slow injection into a vein in which circulation is rapid is indicated—the solution being thereby rapidly diluted and swept away.

### 3 EFFECT OF HYPERTONIC SOLUTIONS ON WALLS OF VEIN

There are certain injections which are given to irritate and damage the wall of the vein, and eventually cause occlusion. This procedure is used to obliterate varicose veins. In these the circulation is very sluggish, and an injection may remain undiluted for a considerable time. For sclerosing (i.e. closing) varicose veins, hypertonic solutions are sometimes used. Solutions of sodium morrhuate and of quinine hydrochloride with urethane have been used for this purpose but, as they have serious side reactions Ethanolamine Oleate Injection is now preferred, being more stable and less toxic.

In injecting strongly hypertonic solutions it is usual to select a vein in which circulation is rapid, in order to prevent undesired damage to the wall of the vein. The median basilic vein is selected for reasons indicated previously.

Quinine Dihydrochloride Injection is a case in point. It is used intravenously in some forms of malaria. It is diluted with ten times its volume of Water for Injection and injected slowly. The diluted injection is hypotonic.

### B INTRATHECALLY

Strict isotonicity is essential for intrathecal injections, for the following reasons—

- The circulation of the cerebrospinal fluid is slow, hence a small volume of a paratonic solution will cause a local disturbance of osmotic pressure.
- The volume of the cerebrospinal fluid is much smaller than that of the blood, hence a com-

paratively small injection will affect the osmotic pressure throughout.

- Disturbance of osmotic pressure of the cerebro-spinal fluid quickly causes headache, vomiting, etc.

### C INTRAMUSCULARLY

There are two classes of intramuscular injection. The first comprises aqueous solutions of medicaments intended to be absorbed quickly, these should be slightly hypertonic to encourage a mild local effusion (exosmosis) of tissue fluids which will promote rapid absorption. The second contains injections that must be absorbed slowly, the intention being to produce a depot in the body from which the medicament will be leached at a steady, but not high, rate. As a result, instead of the body fluids containing an unnecessarily high level of drug for a short time (e.g. 4 or 5 hr) they will have a lower, but adequate, level for much longer (e.g. days or weeks). To achieve this, the drug must be either in an insoluble form (e.g. in aqueous suspension) or in solution or suspension in a hydrophobic vehicle (e.g. in an oil). The many different forms and formulations of penicillin strikingly illustrate this form of treatment, which is called depot medication. Aqueous preparations for depot therapy should not be made hypertonic because this would hasten absorption.

### D INTRACUTANEOUSLY

The intracutaneous route is often used for preparations capable of showing if an individual is immune to certain infectious diseases such as diphtheria, scarlet fever and tuberculosis. At a suitable time after injection the site is examined for the presence or absence of inflammation and a diagnosis is made accordingly. To avoid a wrong conclusion it is most important that the active principle of the injection should be the only possible cause of the reaction. Since paratonic solutions might irritate the skin, diagnostic intradermal injections are usually made isotonic. This subject is dealt with more fully in Cooper and Gunn (1957b).

### E SUBCUTANEOUSLY

The volume of these is usually very small, hence a wide range of paratonicity is permissible without fear of untoward effect.

## GENERAL PRINCIPLES FOR ADJUSTMENT TO ISOTONICITY

From what has been stated above, certain general rules can be laid down regarding the desirability of adjusting paratonic solutions. These rules are—

### Solutions for Intravenous Injection

Approximate isotonicity is always desirable. All hypotonic solutions should be rendered isotonic.

Hypertonic solutions produce no appreciable ill effects when injected correctly

#### *Solutions for Subcutaneous Injection*

Isotonicity, although desirable, is not essential, because they are injected into fatty tissue and not into the blood stream.

#### *Solutions for Intramuscular injection*

Aqueous solutions should be slightly hypertonic to promote rapid absorption. Aqueous depot formulations should be isotonic, a hypertonic vehicle might hasten absorption

#### *Solutions for Intracutaneous injection*

Diagnostic preparations should be isotonic since a paratonic solution might cause a false reaction

#### *Solutions for Intrathecal Injection*

These must be isotonic. The volume of the cerebro-spinal fluid is only 60–80 ml, hence a small volume of a paratonic solution will disturb the osmotic pressure and may cause vomiting and other effects

Study of the official injections shows that, usually, the above-mentioned principles have been followed. The intracutaneously administered diagnostic preparations, Schick and Dick Test Toxins are made isotonic. Injections given in small volumes, sub-

cutaneously, intramuscularly or intravenously, are not adjusted, e.g. Atropine Sulphate and Picrotoxin Injections. In other cases where small volumes are injected, particularly by the intramuscular and intravenous routes, the solutions are hypertonic. However, where relatively large volumes are administered adjustment to isotonicity with blood plasma is made, e.g. Sodium Chloride Injection, Sodium Lactate Injection and Compound Injection of Sodium Lactate and, when no strength is prescribed, Sodium Chloride and Dextrose Injection and Dextrose Injection

#### *Isotonic Solutions for Topical Application*

Aqueous solutions applied within the nostrils and also to areas of broken skin may prove irritant if paratonic and, therefore, it is desirable to make them isotonic with blood plasma

#### *Isotonic Solutions for Ophthalmic Use*

It has been generally accepted in the past that tear secretion has an osmotic pressure equivalent to that of a 1.4 per cent solution of sodium chloride. It has been shown (see p. 257) that this is not so but that it has a value identical with that of blood serum. Solutions which are required to be isotonic with the lacrimal secretion should, therefore, be made isotonic with 0.9 per cent sodium chloride solution.

### CALCULATIONS FOR SOLUTIONS ISOTONIC WITH BLOOD

#### 1. Based on Freezing point Data

Certain physical properties of solutions (called colligative properties) are independent of the nature of the dissolved substance—they depend solely on the number of *ultimate units* into which the substance breaks up in solution. These units are molecules in the case of substances which do not ionise, ions when the substance ionises, and a mixture of molecules and ions when the substance ionises partially.

The two colligative properties of interest here are osmotic pressure and depression of freezing point. These, being proportional to the number of units present in the solution, are proportional to one another, and the measurement of one is therefore an indirect measurement of the other. Determination of the depression of freezing point is simpler and more accurate than direct measurement of osmotic pressure, and is therefore used to compare osmotic pressures.

The temperature at which blood plasma freezes is  $-0.52^{\circ}\text{C}$ , i.e. the dissolved substances which it contains depress the freezing point  $0.52^{\circ}\text{C}$  below that of pure water, and any other solution which

freezes at  $-0.52^{\circ}\text{C}$  will have the same osmotic Pressure as blood plasma

It is evident that all hypotonic solutions have a freezing-point higher than that of blood plasma for instance, a 1 per cent w/v solution of procaine hydrochloride freezes at  $-0.122^{\circ}\text{C}$ . In order to render this solution isotonic an adjusting substance must be added to reduce the freezing point to  $-0.52^{\circ}\text{C}$ . The proportion of adjusting substance required is that which will produce a solution with a freezing point of  $-0.398^{\circ}\text{C}$ , i.e. the difference between  $-0.52$  and  $-0.122^{\circ}\text{C}$ . As a general statement, the proportion of adjusting substance required is that which will lower the freezing point by—

$$0.52^{\circ}\text{C} - \text{the freezing point depression of the unadjusted solution}$$

This value will vary, and a table showing every value would be cumbersome, moreover it is unnecessary because the freezing points of solutions of the same substance, provided they are fairly dilute and not very unequal in strength are proportional to their strength. Thus the freezing point of a 0.5

per cent w/v of procaine hydrochloride may be taken as  $0.061^{\circ}\text{C}$ , i.e. half that of a 1 per cent solution. From Table 20.3 it will be seen that a 1 per cent w/v solution of sodium chloride depresses the freezing point  $0.576^{\circ}\text{C}$ . Selecting sodium chloride as the adjusting substance for the 1 per cent w/v solution of procaine hydrochloride mentioned above, the proportion needed will be

$$\frac{0.398}{0.576} \text{ per cent} = 0.69 \text{ per cent}$$

The general formula for calculation for solutions

to be rendered isotonic with blood serum is therefore—

Percentage w/v of Adjusting Substance needed =  $\frac{0.52 - a}{b}$  where  $a$  is the figure representing the freezing point of the unadjusted solution, and  $b$  is the figure representing the freezing point of a 1 w/v solution of the adjusting substance

Each solute exerts its effect on the freezing point, independent of the others present. Hence if two or more substances are present,  $a$  is the sum of their depressions

Table 20.3

The Freezing point of a 1 per cent w/v Solution of Substances Commonly used in Injections, Ophthalmic Solutions, and for Adjustment

Adrenaline Acid Tartrate	-0.098°C	Morphine Hydrochloride	-0.086°C
Amethocaine Hydrochloride	-0.109°C	Morphine Sulphate	-0.079°C
Apomorphine Hydrochloride	-0.080°C	Neostigmine Bromide	-0.127°C
Atropine Methonitrate	-0.100°C	Neostigmine Methylsulphate	-0.115°C
Atropine Sulphate	-0.074°C	Papaverine Hydrochloride	-0.061°C
Boric Acid	-0.288°C	Pethidine Hydrochloride	-0.125°C
Calcium Chloride, $6\text{H}_2\text{O}$	-0.200°C	Phenobarbitone Sodium	-0.135°C
Calcium Gluconate	-0.091°C	Physostigmine Salicylate	-0.090°C
Carbachol	-0.205°C	Philocarpine Nitrate	-0.132°C
Chlorbutol	-0.138°C	Potassium Chloride	-0.439°C
Cocaine Hydrochloride	-0.090°C	Procaine Hydrochloride	-0.122°C
Copper Sulphate	-0.10°C	Quinine Hydrochloride	-0.077°C
Dextrose	-0.10°C	Silver Nitrate	-0.190°C
Emetine Hydrochloride	-0.058°C	Silver Proteinate Mild	-0.095°C
Ephedrine Hydrochloride	-0.165°C	Sodium Benzoate	-0.23°C
Ethanolamine	-0.300°C	Sodium Bicarbonate	-0.38°C
Fluorescein Sodium	-0.181°C	Sodium Chloride	-0.576°C
Homatropine Hydrobromide	-0.097°C	Sodium Nitrate	-0.48°C
Hyoscine Hydrobromide	-0.068°C	Sulphacetamide Sodium	-0.132°C
Lignocaine Hydrochloride	-0.130°C	Tryparsamide	-0.113°C
Lobeline Hydrochloride	-0.091°C	Urethane	-0.178°C
Magnesium Sulphate	-0.094°C	Zinc Sulphate	-0.086°C

## EXAMPLE 20.1

What proportion of Procaine Hydrochloride will yield a solution isotonic with blood plasma?

Table 20.3 shows that the freezing point of a 1% w/v solution of procaine hydrochloride is  $-0.122^{\circ}\text{C}$ .

percentage w/v of Procaine Hydrochloride required

$$= \frac{0.52 - 0.00}{0.122} = 4.6\% \text{ w/v}$$

**EXAMPLE 20 2**

*Find the proportion of sodium chloride required to render a 1 per cent solution of Cocaine Hydrochloride isotonic with blood plasma*

Table 20 3 shows that the freezing point of a 1% w/v solution of cocaine hydrochloride is  $-0.09^{\circ}\text{C}$  and that of a 1% w/v solution of sodium chloride is  $-0.576^{\circ}\text{C}$

percentage w/v of sodium chloride required

$$= \frac{0.52 - 0.09}{0.576} = 0.746\% \text{ w/v}$$

**EXAMPLE 20 3**

*Find the proportion of sodium chloride required to render a 1.5 per cent solution of Procaine Hydrochloride isotonic with blood plasma*

Table 20 3 shows that the freezing point of a 1% w/v solution of procaine hydrochloride is  $-0.122^{\circ}\text{C}$ , and that of a 1% w/v solution of sodium chloride is  $-0.576^{\circ}\text{C}$ .

percentage w/v of sodium chloride required

$$= \frac{0.52 - (0.122 \times 1.5)}{0.576} = 0.585\% \text{ w/v}$$

Sometimes a solution has to be prepared of such a strength that, on dilution with a specified volume of water, it will be isotonic with lacrimal secretion

This may be calculated in three different ways as in Example 20 4

**EXAMPLE 20 4**

*Find the amount of sodium chloride necessary to be included in 100 ml of a 0.3 per cent solution of zinc sulphate so that, on dilution with an equal quantity of water, it will be isotonic with lacrimal secretion*

A little consideration will show that it is incorrect to work out the quantity of sodium chloride as in the previous examples and then double the answer. Isotonicity with lacrimal secretion would be achieved only if the quantity of medicament were also doubled, and this is not intended.

(i) The simplest method is to base the calculation on twice the freezing point depression for lacrimal secretion, when the general formula becomes

$$\text{Percentage of adjusting substance} = \frac{(2 \times 0.52) - a}{b}$$

Table 20 3 shows that the freezing point of a 1 per cent w/v solution of zinc sulphate is  $-0.086^{\circ}\text{C}$  and that of a 1 per cent w/v solution of sodium chloride is  $-0.576^{\circ}\text{C}$

Percentage of sodium chloride required

$$= \frac{1.04 - (0.3 \times 0.086)}{0.576} = 1.76$$

Therefore, in 100 ml there will be 1.76 G

(ii) Base the calculation on the diluted solution, i.e. instead of 100 ml of a 0.3 per cent solution calculate the amount of sodium chloride necessary for 200 ml of a 0.15 per cent solution

$$= \frac{0.52 - (0.15 \times 0.086)}{0.576} = 0.88 \text{ per cent}$$

0.88 per cent of 200 ml = 1.76 G

(iii) Calculate in the normal way ignoring the dilution and add to the result 0.9 G of sodium chloride, i.e. 0.9 per cent of 100 ml

$$\frac{0.52 - (0.3 \times 0.086)}{0.576} = 0.86 \text{ per cent}$$

$$\begin{aligned} 0.86 \text{ per cent of } 100 \text{ ml} &= 0.86 \text{ G} \\ 0.86 \text{ G} + 0.9 \text{ G} &= 1.76 \text{ G} \end{aligned}$$

These three methods can easily be adapted for triple or any other dilutions which may be prescribed

## 2 Based on Molecular Concentration

The term molecular concentration indicates the number of units, i.e. molecules or ions, or both, present in a solution. As stated above, osmotic pressure is directly proportional to the number of these units, in other words, it is proportional to the molecular concentration.

If one grammie-molecule of any non ionising substance is dissolved in 100 G of water the solution may be said to have a grammie-molecular concentration of 1 per cent. For example, the molecular weight of urea is 60, and therefore a solution of 60 G of urea in 100 G of water may be said to have a molecular concentration of 1 per cent.

An aqueous solution which has a molecular concentration of 1 per cent\* depresses the freezing-point to  $-18.6^\circ\text{C}$ . As stated earlier, blood plasma freezes at  $-0.52^\circ\text{C}$ , hence the molecular concentration of blood plasma can be found by comparison of these figures thus—

A depression of  $18.6^\circ\text{C}$  is given by a molecular concentration of 1 per cent

a depression of  $0.52^\circ\text{C}$  is given by a molecular concentration of  $\frac{1 \times 0.52}{18.6} = 0.030$  per cent, approx

Hence a solution which has a molecular concentration of 0.030 per cent, i.e. has 0.030 grammie-

\* The determinations are made with dilute solutions the stated depression of  $18.6^\circ\text{C}$  being calculated therefrom

molecules in 100 G of solvent, is isotonic with blood plasma. As these solutions are very dilute and aqueous, the concentration may be considered as 0.030 grammie-molecules in 100 ml.

To convert grammie-molecules to grammes it is necessary to multiply by the molecular weight. For example, the molecular weight of dextrose is 180, therefore a solution which contains 0.030 per cent  $\times 180 = 5.4$  per cent is isotonic with blood plasma.

The formula for calculation for solutions isotonic with blood plasma with non-ionising substances is therefore—

$$\begin{aligned} \text{Per cent w/v of substance required} \\ = 0.030 \times \text{gramme-molecular weight} \\ \text{of the substance} \end{aligned}$$

The grammie-molecular weight of a substance which ionises completely yields one grammie-ion for each ion in the molecule. Hence if the grammie-molecular weight of a substance which yields 2 ions is dissolved in 100 G of water, there will be present 2 grammie ions, i.e. the molecular concentration is 2.0 per cent—the term molecular concentration including both grammie-molecules and grammie ions.

The formula for calculation for solutions isotonic with blood plasma with substances which ionise is therefore—

$$\begin{aligned} \text{Per cent w/v of substance required} \\ = \frac{0.03 \times \text{gramme molecular weight of the substance}}{\text{number of ions into which substance dissociates}} \end{aligned}$$

### EXAMPLE 205

Find the proportion of dextrose needed to form a solution isotonic with blood plasma

The molecular weight of dextrose is 180, and it is non ionising

$$0.030 \times 180 = 5.4\% \text{ w/v is required}$$

(The B.P. uses 5%)

**EXAMPLE 20 6**

*Find the proportion of sodium chloride required to form a solution isotonic with blood plasma*

The molecular weight of sodium chloride is 58.5, and it dissociates into 2 ions

$$\frac{0.030 \times 58.5}{2} = 0.88\%$$

(The B.P. uses 0.90%)

---

**EXAMPLE 20 7**

*Find the proportion of boric acid required to form a solution isotonic with lachrymal secretion*

The molecular weight of boric acid is 62, it is practically non ionising  
 $0.030 \times 62 = 1.86\%$

---

Solutions containing a medicament are made isotonic by adding sufficient of a suitable substance (e.g. sodium chloride, dextrose) to procure a total concentration of 0.030 per cent gramme molecules

**EXAMPLE 20 8**

*Find the proportion of sodium chloride required to render a 1 per cent solution of cocaine hydrochloride isotonic with blood plasma*

The molecular weight of cocaine hydrochloride is 339.5, it yields 2 ions

The gramme molecular concentration of a 1 per cent solution is therefore—

$$1 \times \frac{2}{339.5} = 0.0059\%$$

The sodium chloride required must, therefore, produce a gramme molecular concentration of  $0.030 - 0.0059 = 0.024\%$

The molecular weight of sodium chloride is 58.5, it yields 2 ions, therefore—

$$\frac{0.024 \times 58.5}{2} = 0.70\% \text{ of sodium chloride must be added.}$$


---

**EXAMPLE 20 9**

*Find the proportion of dextrose required to render a 1 per cent solution of cocaine hydrochloride isotonic with blood plasma*

From Example 20 8 above, the dextrose required must produce a gramme molecular concentration of 0.024 per cent. The molecular weight of dextrose is 180, it is non ionising

$$0.024 \times 180 = 4.3\% \text{ of dextrose must be added.}$$


---

**EXAMPLE 20 10**

*Give the formula needed for 40 ml of a 2 per cent isotonic solution of Procaine Hydrochloride containing 0.5 per cent of phenol*

The molecular weight of Procaine Hydrochloride is 272.6, and it yields 2 ions

The gramme-molecular concentration of a 2 per cent solution is therefore—

$$2 \times \frac{2}{272.6} = 0.01468\%$$

The molecular weight of phenol is 94, it is practically non ionising. The grammie-molecular concentration of a solution is therefore—

$$\frac{0.5}{94} = 0.0053\%$$

The sodium chloride required must therefore produce a grammie-molecular concentration of 0.030 — (0.01468 + 0.0053) = 0.010%.

The molecular weight of sodium chloride is 58.5, it yields 2 ions

$$\frac{0.0100 \times 58.5}{2} = 0.29\% \text{ of sodium chloride must be added}$$

The formula for 40 ml of solution is, therefore—

Procaine Hydrochloride	0.800 G
Sodium Chloride	0.116 G
Phenol	0.200 G
Water	to 40.000 ml

### Comparison of Methods 1 and 2

Errors occur in both methods of calculation, particularly when ionising substances are involved because the laws on which these calculations are based hold only at infinite dilutions. In more concentrated solutions osmotic pressure and freezing point depression are not strictly proportional to the concentration, and the discrepancy increases with the concentration. The possibility of error is reduced to some extent in the freezing point method, which utilises experimental data for concentrations approaching isotonicity, and therefore yields results more nearly correct than those obtained by the other method. It may be added that the osmotic pressure of a solution of the composition given by calculations by either method will rarely differ from that of blood serum by more than 10 per cent.

The freezing point method is to be preferred when the requisite data are available. The value of the alternative method is that it demands only a knowledge of the molecular weight of, and the number of ions yielded by, the substance involved. The method fails when the molecular weight is unknown, as for colloids, e.g. silver proteinate.

In considering whether the errors of either method are of much importance, it should be remembered that haemolysis does not occur with solutions which are approximately half isotonic or stronger (see p. 247). It follows therefore that small errors in either method are of no practical significance.

### 3 Based on Comparison with Isotonic Solution of Sodium Chloride

The formula for this method for solutions isotonic with blood plasma is—

$$\checkmark \frac{\text{Percentage of substance required}}{= \frac{0.9 \times 2}{58.5} \times \frac{\text{molecular weight of substance}}{\text{number of ions yielded}}}$$

Molecular concentration forms therefore the basis of this method, thus the formula may be stated—

$$\frac{\text{Percentage of substance required}}{= \frac{\text{molecular concentration deduced from a } 0.9 \text{ per cent solution of sodium chloride}}{\times \frac{\text{molecular weight of substance}}{\text{number of ions yielded}}}}$$

In this method again the basis is a figure determined experimentally at a concentration exactly isotonic. Thus if the osmotic pressures of solutions of the substance concerned show the same divergence from those calculations as does sodium chloride, the anomalies will cancel themselves out, and the method will give a fairly accurate result. As a rule, with univalent salts, i.e. those resembling sodium chloride, this is approximately true with other electrolytes and *non-electrolytes*, errors are introduced, but, as before, they may be ignored.

Methods 2 and 3 may be compared thus—

#### (2) Molecular Concentration Method

$$\text{True molecular concentration} \times \frac{\text{molecular weight of substance}}{\text{number of ions yielded}}$$

→ *Result* a slightly hypotonic solution

#### (3) Equilibration Method

$$\text{False high molecular concentration} \times \frac{\text{molecular weight of substance}}{\text{number of ions yielded}}$$

→ *Result* a solution less hypotonic than the above and in some cases slightly hypertonic

**EXAMPLE 20 11**

*Find the weight of sodium sulphate required for 4 ounces of solution isotonic with blood plasma*

Molecular weight of sodium sulphate = 322

Molecular weight of sodium chloride = 58.5

Sodium sulphate yields three ions ( $\text{Na}^+$ ,  $\text{Na}^+$ ,  $\text{SO}_4^{2-}$ )

percentage of Sodium Sulphate required

$$= \frac{0.9 \times 2^*}{58.5} \times \frac{322}{3} = 3.3\%$$

The quantity required for 4 ounces is therefore 58 grains (approx.)

**EXAMPLE 20 12**

*Give a formula for 8 ounces of a solution containing  $\frac{1}{2}$  grain of morphine hydrochloride in 5 minims rendered isotonic with blood plasma using sodium chloride*

For the purpose of comparison this example is worked out by all three methods—

**METHOD 1**

*Data required—*

A 1% solution of morphine hydrochloride depresses the freezing point 0.086°C

A 1% solution of sodium chloride depresses the freezing point 0.576°C  
5 minims of this solution contains  $\frac{1}{2}$  grain

✓ 110 minims of this solution contains 2.75 grains

The solution therefore contains 2.75 percent of morphine hydrochloride  
percentage of sodium chloride required

$$= \frac{0.52 - (0.086 \times 2.75)}{0.576} = \frac{0.284}{0.576} = 0.49\%$$

**METHOD 2**

*Data required—*

Molecular weight of morphine hydrochloride = 375.7, it yields 2 ions  
Molecular weight of sodium chloride = 58.5, it yields 2 ions

As found above, the solution contains 2.75 per cent of morphine hydrochloride  
The molecular concentration of a 2.75 per cent solution is therefore

$$\frac{2.75 \times 2}{375.7} = 0.0146$$

The sodium chloride must therefore produce a molecular concentration of  $0.030 - 0.0146 = 0.0154$

percentage of sodium required

$$= \frac{0.0154 \times 58.5}{2} = 0.45\%$$

**METHOD 3**

*Data required—*

As shown under Method 2

Percentage of sodium chloride required

$$= \left( \frac{0.9 \times 2}{58.5} - \frac{2 \times 2.75}{375.7} \right) \times \frac{58.5}{2} = 0.47\%$$

\* Notice that this value is 0.030 i.e identical with the figure representing the molecular concentration (as a percentage) in Method 2

The three formulae will be—

	Method 1	Method 2	Method 3
Morphine Hydrochloride	96 gr	96 gr	96 gr
Sodium Chloride	17 15 gr	15 75 gr	16 45 gr
Water	to 8 oz	8 oz	8 oz

All three are, therefore, in sufficiently close agreement for the purpose in view

In chapter 12 there is an account of methods used for the rapid adjustment of ophthalmic solutions to isotonicity with lachrymal secretion in extemporaneous dispensing

#### 4 Graphical Method Based on Vapour Pressure and Freezing Point Determinations

The accuracy of the figures in the *British Pharmaceutical Codex* and other reference books throughout the world is due in large measure to the excellent work of Pedersen Bjergaard and his colleagues (1950). In particular, they found that the osmotic pressure of lachrymal secretion was, for all practical purposes, the same as that of blood plasma, whereas it had been generally accepted that the osmotic pressure of lachrymal secretion was equivalent to that of a 1 4 per cent solution of sodium chloride.

Their method for obtaining accurate data and for calculating quantities for the adjustment of solutions is outlined briefly below.

A series of solutions of sodium chloride covering a suitably wide range was prepared. The freezing points of these solutions were determined with great accuracy, in particular avoiding the error due to the separation of ice, i.e. the freezing of water rather than solution. These solutions were used as standards.

Comparison of solutions of many common medicaments with these standards was made using a vapour pressure method. This was done by using a special thermocouple of constantan and manganin as illustrated in Fig 206. Each loop of the thermocouple holds a drop of liquid. A drop of medicament solution was placed in one loop and a drop of a standard solution in the other. The thermocouple enclosed in a brass container, was connected to a sensitive galvanometer. Since differences in the vapour pressure of the two drops give differences in the rate of evaporation and, therefore, of cooling such differences are indicated thermoelectrically by a deflexion of the galvanometer needle. Two solutions with the same vapour pressure will give no deflexion and have, therefore, the same vapour pressure and freezing point. By trial and error the standard solution with the nearest vapour pressure to that of the medicament solution may be found.

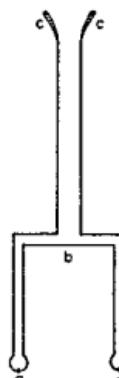


Fig 206 THERMOCOUPLE ELEMENT

a—thermocouples ab—Constantan wire ac—Manganin wire

An accurate graph plotting freezing point against percentage concentration, was prepared for each medicament and on the same graph the curve for the adjusting substance. From these curves it is easy to find the percentage of adjusting substance necessary to render any particular percentage of medicament isotonic with blood plasma.

An ingenious method for reading from the graph the required percentage of adjusting substance was devised. A mirrored curve of the adjusting substance (usually sodium chloride) was added to the graph and the method of reading the result is shown in Fig 207 in which a 2 per cent solution of medicament M is to be made isotonic with blood plasma using sodium chloride as the adjusting substance.

The graph, covers the range of temperature 0°C to -0 52°C (the freezing point of blood plasma). It will be seen that a 2 per cent solution of medicament M has a freezing point of -0 3°C. Therefore a further depression of 0 22°C must be given by the sodium chloride. This could be found by taking a

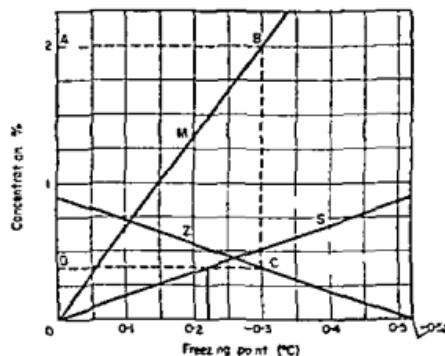


Fig. 207

vertical line from 0.22°C to cut the sodium chloride curve, but it will be noticed that this is on the same horizontal as the point where the vertical from 2 per cent on the medicament curve cuts the mirrored sodium chloride curve.

Thus to find the percentage of adjusting substance necessary to render a 2 per cent solution of medicament *M* isotonic with blood plasma it is only necessary to follow the dotted line *ABCD* and read the result, in this case 0.38 per cent.

#### 5 International Pharmacopoeia Method

The quantities of adjusting substances required to render hypotonic solutions of medicaments isotonic

with tissue, blood or lacrimal secretion are obtained from a series of charts, 67 in number, and occupying 23 pages of the *Supplement to the First International Pharmacopoeia*.

The absolute simplicity of the method is indicated by the following example from the *Supplement*

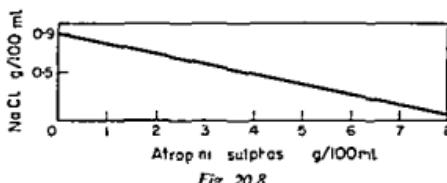


Fig. 20.8

The method is as follows:

Note the point on the abscissa corresponding to the percentage of medicament. Find the ordinate of the curve corresponding to this abscissa. This is the percentage of sodium chloride required. The percentage may also be read from a table provided with the chart.

When more than one medicament is involved find the percentage of sodium chloride necessary for each in the manner described above and use the following formula

$$\frac{1}{n} \times 0.9 = \% \text{ w/v of sodium chloride required.}$$

In the great majority of cases sodium chloride is the adjusting substance. In a few cases potassium nitrate is used, e.g. for silver nitrate.

## THE NEED FOR A PRESERVATIVE

### BACTERICIDES IN MULTI DOSE INJECTIONS

Solutions for injection are frequently dispensed in containers holding several doses. These are known as multi-dose injections and are supplied in bottles closed with some form of rubber cap. The purpose of the latter is to allow the withdrawal of single doses of the injection with minimal risk of contaminating the remainder. Usually, the required dose is withdrawn in the following manner—

1 The surface of the rubber cap is swabbed with an antiseptic solution, e.g. 75 per cent v/v ethanol (or preferably isopropanol), or Weak Iodine Solution B.P. (Medical Research Council, 1962). The bottle is then held inverted; this allows the needle to reach the whole of the contents and largely prevents aerial bacteria from falling on to the rubber cap.

2 The sterile needle of a sterile syringe is plunged

through the cap and the dose withdrawn. The withdrawal of liquid from a rubber-capped bottle causes a reduction of pressure inside and the atmospheric pressure outside tends to force the piston towards the nozzle. To overcome this, it is a common but objectionable practice to inject into the bottle about as much air as liquid to be withdrawn, thus equalising the pressure within and without and allowing free movement of the piston, with no risk of sudden influx of air when the needle is withdrawn. See 'Aseptic Technique' for further discussion of this procedure.

3 The needle is withdrawn, the bottle returned to the upright position and the cap again swabbed.

However, the above technique does not eliminate contamination from the following causes—

I Failure in the sterilisation of the cap, from using

- an ineffective antiseptic or not allowing sufficient time for bactericidal action
- 2 Bacteria settling on the cap
  - 3 Failure in the sterilisation of the needle or syringe
  - 4 Injection of unsterile air
  - 5 Inrush of unsterile air as the needle is withdrawn

The real danger in introducing bacteria into an injection is the rapidity with which they can multiply in a suitable medium. The last dose from a bottle might easily contain millions of organisms and the *British Pharmacopœia* therefore states that 'When the container is sealed to permit the withdrawal of successive doses on different occasions, the solution or preparation of the drug contains sufficient of a suitable bactericide.'

Although the bactericides used in multiple dose containers destroy vegetative bacteria in a few hours at room temperature they are not usually sporicidal i.e. capable of killing bacterial spores. However, spores are unlikely to develop in their presence (even if this occurred the vegetative forms would soon be destroyed) and, therefore, the number will remain small and is unlikely to prove dangerous. The body has natural defences against bacterial invasion and these break down only when the bacteria are numerous or of high virulence.

Another reason for preventing the growth and multiplication of micro-organisms in multi-dose injections is the destructive action that some bacteria and moulds have on certain medicaments. For example, penicillin is rapidly inactivated by penicil lase an enzyme secreted by a number of air borne bacteria.

#### BACTERICIDES IN INJECTIONS STERILISED BY FILTRATION

Sterilisation by filtration requires a very high degree of manipulative skill to prevent accidental contamination of the solution. Consequently, when this method is used a number of containers from each completed batch must be tested for sterility. But, even if these are sterile, there is a far from negligible possibility that some of the rest of the batch may be contaminated (see 'Sterility Testing') and to cover this risk the *British Pharmacopœia* allows (it is not obligatory) the addition of a suitable bactericide to the solution before filtration. If this precaution is taken it is important to remember that single-dose injections will also contain the bactericide, this will be reflected in the labelling requirements and the design of sterility tests for the product.

#### BACTERICIDES SUITABLE FOR AQUEOUS PREPARATIONS

The desirable features of a suitable preservative for a multiple dose injection have been summarised by Sykes (1958),

- 1 Ability to prevent the growth of, and preferably to kill, contaminating organisms
- 2 Compatibility with the medicament, even on long storage
- 3 Low absorption rate into rubber (see containers and closures)
- 4 Absence of toxicity to the patient in the quantities employed in the injection

The *British Pharmacopœia* suggests Phenol 0.5 per cent, Cresol 0.3 per cent, Chlorocresol 0.1 per cent and Phenylmercuric nitrate 0.001 per cent (In all cases the percentages are w/v). The *International Pharmacopœia* allows phenylmercuric borate as an alternative to the nitrate, it is much more soluble (1 in 25) than the nitrate (1 in 1,500). This list provides a choice that allows for incompatibilities with medicaments but all four have disadvantages. For example, in the concentrations recommended, only chlorocresol and phenylmercuric nitrate are effective against mould spores, while phenol is the only one that is not appreciably absorbed by rubber. However, these substances are only suggestions and others may be substituted if they are more satisfactory in particular cases and their bactericidal activity is at least equivalent to that of 0.5 per cent phenol. Thiomersal 0.01-0.02 per cent is used in certain immunological preparations while procainamide and some commercial cortisone injections contain benzyl alcohol 0.9 per cent (see Gershenson, 1952).

A preservative is unnecessary in multi-dose injections prepared by heating with a Bactericide because these already contain a lethal substance, nor is one necessary when the medicament has bactericidal power as in the Codex Injections of Leptazol and Ethanolamine Oleate. The latter also contains 2 per cent of benzyl alcohol as a local anaesthetic and, therefore, even if the active ingredient had no antibacterial activity, there would be no need for an additional bactericide.

(For further information see Davis, 1948, and Hartshorn, 1953.)

#### BACTERICIDES SUITABLE FOR OILY PREPARATIONS

The bactericides mentioned in the previous section are more soluble in fats and oils than in water and, therefore, when present in an aqueous liquid containing bacteria will tend to leave the water and

concentrate in the lipid constituents of the bacteria, if sufficient is absorbed the organisms will be inhibited or killed.

Oily solutions of these substances would be expected to have lower antibacterial activity because the bactericides will not leave the oil as readily as they will leave an aqueous medium, i.e. the partition coefficient (see Cooper and Gunn, 1957c) is much less in favour of the bactericide concentrating in the bacteria.

The fact that the B.P. recommends as bactericides for oily preparations, phenol, cresol and chlorocresol in exactly the same concentrations as for aqueous products is not a failure to take their reduced activity into account but a reflection of the difficulty of investigating the efficiency of bactericides in oily media. To study this problem it is necessary to test oils for living organisms and, as will be discussed later, reliable sterility tests on oils are very difficult to design. The official attitude is that although Bactericides are ineffective in oils, their addition affords protection against contamination with vegetative bacteria. It is fortunate, perhaps, that micro-organisms are unlikely to multiply in an anhydrous environment.

Eisman, Jaconis and Mayer (1953) studied the sporicidal activities in sesame oil of a number of substances, including commonly used preservatives. Since a phenylmercuric salt even at 0.01 per cent, was one of the least active compounds tested, it is understandable that phenylmercuric nitrate is not in the list suggested in the Pharmacopoeia.

#### BACTERICIDES IN INTRAVENOUS INJECTIONS

If bactericides were added to intravenous injections of large volume there would be a risk of administering a toxic dose. Therefore, they must not be included if the dose exceeds 15 ml. As a result, bactericidal agents cannot be used in infusion fluids and, consequently, from the official statement quoted earlier, these cannot be packed in multiple-dose containers. Each container must contain one dose only, and suitable types are discussed in the next chapter. The user of an intravenous solution packed in such a container might be tempted, if any remained after the first infusion, to administer it on another occasion. This would be dangerous, because bacteria that entered during the first time of use would, in the absence of a bactericide, multiply rapidly on storage. A subsequent injection given from the container might harbour a very large number of viable organisms. After withdrawal of the required dose, any unused portion should be rejected, and it is sound practice to draw the attention

of the user to this necessity by attaching a special label to the container, e.g. 'Warning Discard any unused portion.' A similar form of labelling is directed for Bernegride Injection B.P. This injection may not contain a bactericide because it is used in large volumes intravenously to counteract barbiturate poisoning.

N.B. There is no objection to the inclusion of a bactericide in an intravenous injection when the dose is not more than 15 ml. (See also American Medical Association Report, 1954.)

#### BACTERICIDES IN INTRATEHICAL, INTRACISTERNAL AND PERIDURAL INJECTIONS

The meninges (i.e. the coats of the spinal cord and brain) are very easily irritated and serious inflammation (aseptic meningitis) may be set up by bactericides (Swift, 1950), therefore, these are never included in intrathecal, intracisternal and peridural injections.

Moreover, because the cerebrospinal fluid provides a very favourable medium for bacterial growth it might be dangerous to inject even a few organisms from a contaminated multi-dose injection.

For these reasons the Pharmacopoeia insists that injections for administration by these three routes must be packed in ampoules, i.e. single-dose containers sealed by fusion of glass. As peridural injections of as much as 30 ml are occasionally given, ampoules of unusually large size may be necessary.

#### BACTERICIDES IN WATER FOR INJECTION

Many injections are prepared by dissolving the medicament in Water for Injection immediately before use (see Appendix 4). Water for this purpose may be packed in:

- (a) *Single-dose Containers* These are essential when the injection is to be given by the intrathecal, peridural or intracisternal routes or, in a dose of more than 15 ml, intravenously.
- (b) *Multiple-dose Containers* A bactericide must be added to these and its nature and concentration must be stated on the label to prevent accidental use of the water for the injections mentioned in the previous paragraph.

#### BACTERICIDES IN INJECTIONS IDENTIFIED OR ASSAYED BY LIGHT ABSORPTION METHODS

Ultra violet absorption measurements are very useful for the identification and assay of parenteral solutions containing organic medicaments. They are increasingly included in official monographs e.g.

those for injections of cyanocobalamin, diphenhydramine, phytomenadione, pyridostigmine and lobeline.

The presence of a bactericide will interfere with the absorption characteristics of the preparation and this must be taken into account when carrying out these determinations.

#### Incompatibilities of Common Bactericides

A bactericide must be compatible with the medicaments and with auxiliary substances present in the solution that it is required to preserve. Consequently, the *British Pharmacopœia* directs that the chosen bactericide 'shall not interfere with the therapeutic efficacy of the drug or cause a turbidity'.

Some of the incompatibilities reported for the four officially recommended bactericides are given in Appendix 3. From these it appears that phenyl mercuric nitrate is comparatively free from obvious incompatibilities and this may be due to the very small percentage used. Chlorocresol is less satisfactory, some of the results are due to salting out of the bactericide by strong solutions of medicaments, others may have been caused by the use of chlorocresol of insufficient purity but a few lead to serious loss of activity of the active ingredient, e.g. that with ergometrine. Phenol and cresol do not have many incompatibilities and are particularly valuable for the majority of protein containing preparations, e.g. immunological products, because, unlike many bactericides, e.g. chlorocresol, their activity is not appreciably reduced in the presence of organic matter. However, they cause loss of anti-

genicity in the adsorbed toxoids, destroy by denaturation preparations which, like Schick Test Toxin, contain only a small amount of active protein (Holt, 1944), and often cause smarting when injected.

#### Summary

#### B.P. bactericides recommended for—

##### *Aqueous vehicles*

Phenol	0.5 per cent w/v
Cresol	0.3 per cent w/v
Chlorocresol	0.1 per cent w/v
Phenylmercuric nitrate	0.001 per cent w/v

Any other substance with a bactericidal activity not less than that of 0.5 per cent w/v phenol.

##### *Oily vehicles*

Phenol	0.5 per cent w/v
Cresol	0.3 per cent w/v
Chlorocresol	0.1 per cent w/v

These are—

##### *Used in*

Multiple dose injections sterilised by any official method

Single dose injections sterilised by filtration

##### *Unnecessary in*

Injections prepared by Heating with a Bactericide  
Injections in which the medicament has bactericidal activity

##### *Prohibited in*

Intrathecal, intracisternal and peridural injections  
Intravenous injections of more than 15 ml dose  
Injections in which serious interference with light absorption determinations would occur

## UNITS IN WHICH FORMULA IS EXPRESSED

#### Milliequivalents

It is sometimes necessary for pharmacists to prepare solutions of electrolytes from formulae expressed in milliequivalents. This is because the biochemist, in determining the electrolyte concentration of a patient's blood, expresses the results in terms of this unit. He records the concentration of each ion in this way and conditions, such as sodium or potassium excess or depletion, may be assessed by the physician. The latter may then prescribe electrolyte solutions to correct any abnormality and he does so in the same units.

#### THE MILLIEQUIVALENT UNIT

It is first necessary to be quite clear about the unit involved before considering how to convert these into weighable quantities.

The equivalent weight of a substance is the atomic

weight divided by the valency. This is expressed in grammes. A milliequivalent (mEq) is one thousandth part of the equivalent weight and is, therefore, the same figure expressed in milligrammes. Table 20.4 makes this clear.

Formulae for electrolyte solutions which the pharmacist may require to prepare state the number of mEq of each anion or cation in a given volume of water, usually one litre. Suitable salts are used to provide these ions.

The quantity of a salt containing 1 mEq of a particular ion is obtained by dividing the molecular weight of the salt by the valency of that ion multiplied by the number of such ions in the molecule.

Weight, in mg of salt containing one mEq =  $\frac{\text{molecular weight of salt}}{\text{valency of ion} \times \text{number of such ions in the molecule}}$

Table 20.5 makes this clear.

Table 20 4

<i>Ion</i>		<i>Atomic weight</i>	<i>Equivalent weight (atomic weight valency)</i>	<i>Weight of 1 mEq (mg)</i>
Sodium	$\text{Na}^+$	23	23	23
Potassium	$\text{K}^+$	39	39	39
Calcium	$\text{Ca}^{++}$	40	20	20
Chloride	$\text{Cl}^-$	35.5	35.5	35.5
Bicarbonate	$\text{HCO}_3^-$	61	61	61
Phosphate	$\text{HPO}_4^{--}$	96	48	48

Table 20 5

<i>Ion</i>	<i>Salt used</i>	<i>M wt of salt</i>	<i>Valency of ion</i>	<i>Number of ions in salt</i>	<i>Weight of salt containing 1 mEq of the ion</i>
$\text{Na}^+$	Sodium chloride	58.5	1	1	58.5 mg
$\text{Na}^+$	Sodium phosphate	358	1	2	179.0 mg
$\text{Ca}^{++}$	Hydrated calcium chloride	219	2	1	109.5 mg
$\text{Cl}^-$	Hydrated calcium chloride	219	1	2	109.5 mg

Table 20 6

<i>Ion</i>	<i>Milliequivalent (mEq) mg</i>	<i>Salt</i>	<i>mg of salt containing 1 mEq of specified ion</i>
$\text{Na}^+$	23.0	Sodium chloride	58.5
$\text{K}^+$	39.0	Sodium bicarbonate	84
$\text{Ca}^{++}$	20.0	Potassium chloride	74.5
$\text{Mg}^{++}$	12.2	Calcium chloride, $\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$	109.5
$\text{Cl}^-$	35.5	Magnesium sulphate, $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$	123
$\text{HCO}_3^-$	61.0	Magnesium chloride, $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$	101.5
$\text{HPO}_4^{--}$	48.0	Sodium chloride	58.5
		Sodium bicarbonate	84
		Sodium phosphate, $\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$	179

The British Pharmaceutical Codex 1959 includes a table showing the weights of salts which contain 1 mEq of specified ions commonly prescribed in this way. Table 20 6 is selected from that table and is followed by an example of its use.

By using Table 20 6 it is extremely simple to calculate the required weights, which Example 20 12 illustrates.

#### Importance of Expressing Plasma Electrolyte Concentration in mEq

From Table 20 7, showing the average normal composition of blood plasma, it will be seen that no balance or relationship is evident when acid and basic ions are expressed in absolute weights. When, however, they are recorded in mEq, it is clear that cations and anions are equally balanced. The

## EXAMPLE 20 12

Sodium Na <sup>+</sup>	147 mEq /litre
Potassium K <sup>+</sup>	4 mEq /litre
Calcium Ca <sup>++</sup>	4 mEq /litre
Chloride Cl <sup>-</sup>	155 mEq /litre
Water for Injection	to 1 litre

Using the table the required quantities of salts are—

Sodium chloride	$147 \times 58.5 \text{ mg} = 8.6 \text{ G}$
Potassium chloride	$4 \times 74.5 \text{ mg} = 0.3 \text{ G}$
Calcium chloride	$4 \times 109.5 \text{ mg} = 0.438 \text{ G}$

It will be obvious that these quantities contain the correct amount of chloride since the anions and cations are equal in number

*Conversion Equations*

To convert quantities expressed in mEq /litre into weighable quantities the following formulae may be used, where  $W$  = the number of milligrammes of salt containing 1 mEq of the required ion and  $E$  = the number of mEq /litre

To convert mEq /litre to mg/litre	$W \times E$
To convert mEq /litre to G/litre	$W \times E - 1,000$
To convert mEq /litre to % w/v	$W \times E - 10,000$

## EXAMPLE 20 13

Express the following formula as percentage w/v—

Sodium	20 mEq /litre
Potassium	30 mEq /litre
Magnesium	5 mEq /litre
Chloride	45 mEq /litre
Phosphate	10 mEq /litre
Water for Injection	to 1 litre

Anions and cations are, of course, equal in number and must be matched in a suitable way. If the phosphate is taken as sodium phosphate, the potassium, magnesium and the remainder of the sodium will be taken as chloride

The formula, therefore, becomes—

Disodium hydrogen phosphate	$10 \times 179.0 - 10,000 = 0.179\% \text{ w/v}$
Sodium chloride	$10 \times 58.5 - 10,000 = 0.059\% \text{ w/v}$
Potassium chloride	$30 \times 74.5 - 10,000 = 0.224\% \text{ w/v}$
Magnesium chloride	$5 \times 101.5 - 10,000 = 0.051\% \text{ w/v}$

It is usual to express the strength of electrolyte solutions as mEq /litre. The number of mEq may, however, be prescribed in any other volume and students should be careful to note the terms employed. Exercise 20 14 is an example

## EXAMPLE 20 14

Prepare 500 ml of an intravenous solution containing 70 mEq of sodium, 2 mEq of potassium, 4 mEq of calcium and 76 mEq of chloride

The number of milligrammes of the various chlorides which contain 1 mEq of the required ions is obtained from Table 20 6 and the formula becomes—

(continued overleaf)

*Example 20.14 continued*

Sodium chloride	70 × 58.5 ~ 1,000 = 4.095 G
Potassium chloride	2 × 74.5 ~ 1,000 = 0.149 G
Hydrated calcium chloride	6 × 109.5 ~ 1,000 = 0.438 G
Water for Injection	to 500 ml

*To Convert Percentage w/v to mEq/litre*

The number of grammes (C) per 100 millilitres is converted to mg/litre by multiplying by 10 000. This, divided by the weight (W) in mg of salt containing 1 mEq, will give the number of mEq/litre

$$\frac{C \times 10,000}{W} = \text{mEq/litre}$$

**EXAMPLE 20.15**

Express 0.9 per cent sodium chloride solution in terms of milliequivalents per litre

$$\frac{0.9 \times 10,000}{58.5} = 154 \text{ mEq/litre}$$

concentrations, thus presented, simplifies the assessment of electrolyte imbalance and its correction.

It can also be seen that although plasma protein is present to the extent of about twenty times that of chloride, its effect on acid base balance is very much less. Thus can be seen by comparing the absolute weights and mEq in Table 20.7

It may also be noted that although sodium is present in lesser amount than chloride it is able to balance all the chloride and other acid radicles as well as some of the protein. The importance of the sodium ion is thus clearly emphasised.

Table 20.7

Average Normal Composition of Blood Plasma

Cations	mEq/litre	mg/100 ml
Na <sup>+</sup>	142	327
K <sup>+</sup>	5	20
Ca <sup>++</sup>	5	10
Mg <sup>++</sup>	3	4
	<u>155</u>	<u>361</u>
Anions		
HCO <sub>3</sub> <sup>-</sup>	27	165
Cl <sup>-</sup>	103	366
HPO <sub>4</sub> <sup>2-</sup>	2	10
SO <sub>4</sub> <sup>2-</sup>	1	5
Organic acids	6	?
Protein	16	7,100
	<u>155</u>	<u>7,646</u>

It is worthy of note that a solution isotonic with blood plasma contains 155 mEq/litre each of anions and cations.

This provides a method for calculating the adjustment of a solution to isotonicity with blood plasma. For example, to prepare a solution containing 40 mEq/litre of potassium chloride and made isotonic with blood plasma by adding a sufficient quantity of sodium chloride.

From Table 20.7 it is seen that 2 × 155 mEq/litre, i.e. 310 are required for isotonicity.

Potassium chloride provides 40 mEq K<sup>+</sup> and 40 mEq Cl<sup>-</sup>

$$\text{Total} = 80 \text{ mEq}$$

Therefore, the required number of mEq to produce isotonicity

$$= 310 - 80 = 230$$

This is provided by 115 mEq of Na<sup>+</sup> and 115 mEq of Cl<sup>-</sup>

Quantities required are—

Potassium chloride 40 × 74.5 = 3 G

Sodium chloride 115 × 58.5 = 6.7 G

Water for injection to 1,000 ml

**Millieosmols** A millieosmol is a measure of the osmotic effect of a milliequivalent. A solution isotonic with blood plasma contains 155 mEq of anions and a similar number of cations per litre and, therefore, contains 310 millieosmols. As in the case of milliequivalents, the adjustment of solutions to isotonicity with blood plasma may be calculated on the number of millieosmols.

**Milliemols (mM)** A milliemole is the ionic weight

expressed in milligrammes. Since a milliequivalent is the equivalent weight expressed in milligrammes—

$$1 \text{ mEq} = \frac{\text{mM}}{\text{valency}}$$

Concentrations in mM/l (millimoles/litre) is used

when a substance is not readily ionised or when the valency varies with pH. The phosphate ion  $\text{HPO}_4^{2-}$  has a valency of 2 but at the pH of blood there is a mixture of monovalent and divalent ions,  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$ . It would, therefore be more correct to consider the valency as 1.8

## HYDROGEN ION CONCENTRATION OF SOLUTION

The *British Pharmacopœia* attaches considerable importance to the pH values of injections. Some solutions are adjusted to a definite pH or to within a pH range and many must lie within certain limits of pH after preparation. The use of buffers to stabilise the pH is often permitted. There are several reasons for these specifications and the following are examples,

### *1 To Increase the Stability of the Preparation*

An unfavourable pH is probably the most important cause of the decomposition of pharmaceutical substances in aqueous solutions and if a preparation is to withstand a heat sterilisation process or have a long shelf life the optimum pH for maximum stability must be provided. Among the many therapeutic agents that can be made much more stable by correct choice of pH are antibiotics (e.g. penicillin and the tetracyclines), alkaloids (e.g. those of ergot), synthetic bases (e.g. adrenaline), vitamins (e.g. cyanocobalamin and ascorbic acid) and polypeptides (e.g. insulin, oxytocin and vasopressin).

### *2 To Minimise Pain, Irritation and Necrosis on Injection*

The fact that some medicaments are most stable in very acid or very alkaline solutions must be balanced against the knowledge that such solutions are painful on injection (Lupton, 1942) and may cause irritation and even necrosis (death) of the tissues. This is especially true if the subcutaneous or intramuscular routes are used, intravenously, if the injection is given slowly, dilution rapidly occurs and the pH effect is further neutralised by the buffering power of the blood plasma.

Consequently, injections must not be more acid or alkaline than stability dictates. In a number of cases an acidic or basic substance is used in the preparation of an injection, e.g. ethylenediamine (Aminophylline Injection) sodium hydroxide (Compound Sodium Lactate Injection) and hydroiodic acid (Iodised Oil Injection), the pH requirements of the *Pharmacopœia* ensure that no significant excess of these substances is present.

### *3 To Help Detect Decomposition*

Decomposition of a medicament in solution is often accompanied by a pH change that can be used to detect deterioration. Failure to observe the *Pharmacopœia* storage requirements such as protection from light and refrigeration, if this led to a pH change, could be detected in this way.

### *4 To Provide Unsatisfactory Conditions for Growth of Micro-organisms*

Although it would be incorrect to suggest that injections have ever been adjusted to a particular pH solely to prevent the growth of micro-organisms it is sometimes possible, when other considerations necessitate a low or high pH, to make use of it for this purpose. For example, Insulin Injection is adjusted to pH 3 to 3.5 for reasons of stability and since solutions of pH values less than 4 are bactericidal to most micro-organisms, it would not be expected to need much additional protection against bacteria accidentally introduced to multiple dose containers. This has been confirmed by Sykes and Hooper (1954) who showed that 0.2 per cent of phenol (cf. 0.5 per cent, the bactericidal concentration recommended in the B.P.) was sufficient to preserve this injection. However, this effect is less marked with moulds, because these can tolerate acid pHs and, therefore, in an injection like Insulin, in which moulds grow readily, it may not be possible to reduce the concentration of preservative to the level suggested by the results for bacteria. The antibacterial activities of Ethanolamine Oleate Injection B.P.C. and Quinine and Urethane Injection (the latter is no longer official), neither of which requires a bactericide in multi-dose containers, are partly due to high and low pH respectively.

### *5 To Enhance Physiological Activity*

This aspect of the importance of pH has been discussed under 'Eye Drops', where it was pointed out that the free bases of ophthalmic salts are more physiologically active than the salts themselves. There is evidence that the same is true for certain alkaloids and synthetic bases when administered as

injections but because these substances are generally more stable at acid pHs it is often difficult to produce satisfactory preparations without using the salts. The problem was demonstrated by Bullock (1938) and Bullock and Cannell (1941) in a study of the stability and activity of procaine in injections of procaine and adrenaline. They investigated the stability of solutions buffered to slight alkalinity because these were said to have the greatest anaesthetic effect (in dentistry), but they found that such preparations were stable for only a few hours, although acid solutions (pH less than 4) could be autoclaved with very little loss of activity. To ensure the use of a solution of maximum physiological activity, they suggested either aseptic mixture of a sterile alkaline buffer with a sterile acid solution of procaine and adrenaline or, preferably, aseptic addition of Water for Injection to a dry sterile mixture of the active ingredients with buffering materials, in both cases, just before use. Probably, the inconvenience of these aseptic procedures is the main reason why an acid solution (see Procaine and Adrenaline Injection B.P.) is still used. To summarise, where maximum physiological activity is shown by neutral or alkaline solutions, while maximum stability is found in acid media, the pH should be kept as high as is compatible with an acceptable level of stability.

#### The pH Adjustment of Injections

##### 1 Using a pH Meter

Although this is the most accurate method and, when available, should be used for checking final pH values, it is often not as convenient as a colorimetric method for following the progress of a pH adjustment.

##### 2 Using a Comparator

This has been described in connexion with the pH adjustment of culture media and, provided sufficient solution is available to permit the wastage of about 5 ml (the volume needed for the Small Comparator), is a very suitable method. Often the back row of tubes will not be necessary, because the majority of parenteral solutions are colourless.

##### 3 Using a Capillitor

During his academic training and in his examination a student is usually concerned with the adjustment of volumes of injections that are unrealistically small compared with the batch volumes normally prepared in hospitals and industry. This causes a further lack of realism in the method of adjustment that he will use, for, while on a large scale quantities

of 5 or 10 ml can be taken for use in comparators such volumes can be used by a student only if he makes an excess quite out of proportion to the quantity that he requires. For example, for a reason that will be discussed later, he would probably prepare 8 ml if asked to send six 1-ml ampoules of Emetine Injection, 5 ml extra for the pH adjustment would not only mean that he would be making almost twice the volume to be issued but, in addition, he would be wasting an equivalent amount of emetine hydrochloride—probably 360 mg, costing about 12s 6d. Consequently, he will use the capillitor because only small volumes are necessary. However, the use of this method for the adjustment of injections has a serious objection, most injections are aqueous solutions of pure chemicals with little or no buffering action (unlike the broths for which the capillitor was recommended earlier) and, therefore, when they are mixed with an equal volume of the indicator solution (see earlier description of method) the pH of the latter will affect the reaction of the mixture. (This is less important in the comparator method, where a relatively small volume of a weaker indicator is used.) The pH values of the indicator solutions are at the half-way points of their working ranges, consequently, if bromocresol green (half way point 4.4) is used to test water of pH 7 the water will seem to be acid, while if thymol blue (half way point 8.8) is used the water will appear alkaline (B.D.H., 1961). Therefore, it is necessary, when using a capillitor, to check the final adjustment, using either an indicator with an overlapping range or a pH meter.

##### 4 Indicator Papers

These are prepared from special mixed indicators and separate papers are available covering each 1.5 step of the pH range from 2.5 to 10. For the reason given under the capillitor they are unsuitable for unbuffered fluids but can be used as a rapid method of following the early stages of an adjustment. Further precautions in the use of this and the capillitor method are discussed in the section describing the preparation of injections requiring pH adjustment.

#### BUFFERS

The pH of a completed injection can be altered by—

- 1 Decomposition of the medicament.
- 2 Leaching of alkali from the glass of the container.
- 3 Extraction of acid or alkaline impurities from the rubber of the closure.

If preparations are stored correctly, decomposition should be small, and if glass containers are carefully selected and rubber closures conscientiously prepared (see chapter 21) they will not be serious sources of pH affecting extractives.

However, there are a few injection medicaments, including several antibiotics, that very rapidly lose activity outside their optimum pH range for stability. For these substances the *British Pharmacopœia* allows or advises the addition of buffers to minimise the effect of traces of acid or alkali from the above sources. Acetates, citrates and phosphates are the principal buffer salts used, borates are too toxic for injections.

#### *Benzylpenicillin Injection*

The optimum pH range for the stability of penicillin solutions is 6.0 to 7.0 but hydrolysis readily occurs in aqueous solutions with the formation of inactive benzylpenicilloic acid. As this is a dibasic acid, while benzylpenicillin is only monobasic, there is a fall in pH which catalyses further hydrolysis, leading to progressively greater acidity and more rapid hydrolysis until all the antibiotic has been destroyed. Decomposition can be retarded by buffering the pH of the solution to 6.0 to 7.0, e.g. with 4.5 per cent w/w sodium citrate, when the life of the injection is prolonged from 7 to 14 days provided it is stored at 2 to 10°C and from 1 to 4 days if it is kept at approaching 20°C, i.e. at room temperature.

(For further information see Carr and Wing (1951), Clapham (1950), Hadgraft, Hopper and Short (1951) and Bacteriostatics Sub Committee Report (1954).)

#### *Stibophen Injection*

Stibophen is an organic compound containing trivalent antimony. In acid solutions it is stable but if the pH becomes even slightly alkaline a yellow colour develops (probably due to oxidation of the

catechol part of the molecule) and the tervalent antimony is oxidised to the pentavalent form. Protection may be given by including a reducing agent, e.g. sodium metabisulphite or ascorbic acid, but an alternative method is to buffer the pH well on the acid side of neutral. The *British Pharmacopœia* injection is buffered at 5.0 to 5.5 with sodium phosphate. (See Robinson and Page, 1942.)

A rather different application of buffers is found in the formulation of certain depot preparations.

#### *Depot Injections of Insulin*

The use of insoluble forms of medicaments to prolong drug action has been mentioned earlier. The effect of protamine zinc-insulin lasts much longer than that of plain insulin because in the former the insulin is in a very insoluble complex from which it is only slowly made available to the body. Because this complex is most insoluble over the very narrow pH range of 6.9 to 7.3 a phosphate buffer is used to prevent significant variations.

Preparations, like protamine-zinc insulin, that contain a protein foreign to the human body (protamine is obtained from a fish), cause unpleasant protein reactions in some patients. Attempts to make a protein-free insoluble form of insulin were unsuccessful until the discovery that the insulin was converted into an insoluble form if an amount of zinc, equivalent to that in protamine zinc-insulin, was added to a solution of insulin in an acetate buffer. Earlier failures were due to the use of citrate or phosphate buffers, zinc has a greater affinity for phosphate and citrate than for insulin and, consequently, in the presence of these ions insufficient zinc is available to combine with the insulin and produce the insoluble zinc insulin complex.

(For general information on buffers see Cooper and Gunn (1957) and Pockel Book, 1960.)

## STABILITY OF THE MEDICAMENT

Adjustment of pH is the most important method of stabilising injections because so many types of decomposition are catalysed by hydrogen and/or hydroxyl ions. Often a pH can be found at which loss of activity is negligible, provided the preparation is packed and stored correctly, but, in some cases, breakdown still occurs, although at a much reduced rate. By investigating the nature and causes of these destructive changes it is sometimes possible to discover additional ways of improving formulae.

For example, many medicaments are destroyed by oxidation, and possible methods of preventing this are the use of Water for Injection free from Dissolved Air (p. 244), the addition of a reducing agent and the replacement of the air in the container with an inert gas. Traces of heavy metals often accelerate destructive changes and sequestering agents, which take up heavy metals into un ionised complexes, have been used occasionally to prevent this kind of catalysis. In addition, in a few cases,

a stabiliser specific for a particular injection may be included. These methods may be used alone or to supplement pH adjustment.

It is important to appreciate that additions to official preparations are not made unless their value is unquestionable. Ideally, a patient should receive only the drug prescribed, and ancillary substances should be considered undesirable unless they are essential for stability. The toxicity, side-effects, stability and efficiency of any proposed additive must be thoroughly investigated before it is used in practice.

### Methods of Stabilisation

#### 1 ADDITION OF A REDUCING AGENT

The most popular reducing agent for injections is sodium metabisulphite. It is used to prevent the decomposition of adrenaline by oxygen in Adrenaline (West, 1945), Procaine and Adrenaline, and Lignocaine and Adrenaline Injections. It retards the development of the green colour produced when apomorphine is oxidised (see 'Alkalinity of Glass') and also the darkening of morphine sulphate in Morphine Sulphate, and Morphine and Atropine Injections (Yeh and Lach, 1961).

The reducing action of dextrose is used in Carbachol Injection and Phenotolamine Injection. Ascorbic acid has been tried in a number of cases, e.g. adrenaline (West, 1947), stibophen (Robinson and Page, 1942) and chlorpromazine (Sabatini, Gulesich and Doerge, 1956), but usually it is not as good a preservative as sodium metabisulphite. Thiourea has been recommended as an antioxidant for ascorbic acid injections.

(Important reviews relating to the use of antioxidants are those by Schroeter, 1961 and Ingold, 1961.)

#### 2. REPLACEMENT OF AIR BY AN INERT GAS

In many injections where oxygen is a serious cause of decomposition improved stability is obtained by replacement of the air in the final containers with an inert gas after distributing the solution or powder. Nitrogen most satisfactorily fulfils the requirements of inertness and availability but, in the past, carbon dioxide was used occasionally, e.g. for adrenaline and apomorphine.

The method may be used alone, as in the injections containing ergot alkaloids (Ergometrine, Ergotamine and Methylergometrine) and in Tubocurarine Injection, or together with one or more reducing agents as in Apomorphine Injection (sodium metabisulphite) and Chlorpromazine Injection (metabisulphite and ascorbic acid).

Ergotamine tartrate and methylergometrine maleate, two of the salts used in the above-mentioned ergot injections, are so sensitive to oxidation that they decompose even in the dry state and must be stored in an atmosphere of nitrogen in sealed tubes.

Yellow fever vaccine is a freeze-dried preparation containing a weakened but living strain of yellow fever virus. When suspended, ready for use, in an aqueous vehicle the viruses are rapidly inactivated by processes involving oxidative changes. Therefore, it has to be stored dry, in sealed containers, from which the air has been displaced by nitrogen that is both sterile (because this preparation, unlike many injections that require air displacement, cannot be subjected to a sterilisation process after filling) and dry (because a very low moisture content is essential for the preservation of the viability of micro-organisms in the freeze-dried condition).

Nitrogen filling is also used for injections of substances (e.g. Aminophylline and Sulphadiazine sodium) that require protection from carbon dioxide (see 'Water for Injection free from Carbon Dioxide').

#### 3 USE OF SEQUESTRING AGENTS

Trace amounts of heavy metal ions often catalyse destructive changes in medicaments, for example, the breakdown of the sulphur-containing ring in benzylpenicillin (copper, lead, mercury and zinc), the oxidation of adrenaline (cupric, ferric, ferrous and chromic), the degradation of thiomersal (cupric) and the decomposition of oxytetracycline (cupric).

Sometimes such effects can be prevented by adding a substance, named a sequestring agent, that will form a soluble co-ordination compound with the metal in which the latter is held in non-ionisable form. Then, the catalytic property of the ion is often, although not always, suppressed.

The agent that has been most widely investigated for this purpose is ethylenediamine tetra-acetic acid (EDTA), usually as its di- or tri-sodium or calcium disodium salts. Improvements in stability have been reported following its use in adrenaline (Roscoe and Hall, 1956) apomorphine, barbiturate, oxytetracycline, benzylpenicillin (Swallow, 1952), sulphadiazine and thiomersal solutions. Sodium hexametaphosphate (well-known as a water-softener) and dimercaprol (used medically in the treatment of arsenic and mercury poisoning) are other examples of sequestring agents that have been investigated for pharmaceutical applications.

The best known example of the use of sequestration in the stabilisation of a parenteral preparation is the inclusion of the calcium disodium salt of EDTA in poliomyelitis vaccine. Thiomersal, which

is present as a bactericide, is unstable in the presence of traces of cupric ions and the products of its breakdown destroy the antigenicity of the vaccine. Inclusion of the sequestering agent protects the thiomersal and, therefore, the activity of the preparation (Davission, 1956).

When sequestering agents are used to prevent metal-catalysed oxidation they are usually more effective if a reducing agent or antioxidant is present.

(For further information see Frost (1956) and Smith, 1959.)

#### 4 INCLUSION OF SPECIFIC STABILISERS

##### (a) Calcium Gluconate Injection

Calcium gluconate is sparingly soluble in cold water (1 in 30) but readily forms a supersaturated solution, an approximately 10 per cent injection (the B.P. strength) can be prepared by dissolving the salt in hot water. As would be expected, this solution tends to deposit crystals, particularly if it has not been carefully filtered (while hot) to free it from foreign particles that can act as nuclei for crystal formation. An injection in which crystals have separated must not be used. To reduce this tendency to crystallise the Pharmacopoeia allows the replacement of not more than 5 per cent of the gluconate by calcium d-saccharate or other suitable, harmless calcium salt (the lactobionate, glucoheptonate and laevulinate have been used successfully). The mechanism of the stabilisation is not fully understood but the formation of readily soluble compounds from the stabiliser and the gluconate has been suggested (March, Sommers and Moore, 1952).

(See also Chakravarty and Jones (1957) and Sengupta and Roy, 1949.)

##### (b) Sodium Bicarbonate Injection

When solutions of sodium bicarbonate are heated the medicament decomposes according to the equation,—



#### SPECIFIC GRAVITY OF THE SOLUTION

This is of importance in spinal anaesthesia. If the upper part of the patient's body is raised by sloping the operating table, solutions of lower specific gravity than the spinal fluid will tend to rise on injection and those of higher specific gravity will tend to sink. On the other hand, the opposite effects will occur if, as is sometimes necessary for operations on the lower part of the body, the patient

If the carbon dioxide escapes from an imperfect or loose closure the reaction will only partly reverse and the residual carbonate may cause harmful effects, such as haemolysis and calcium precipitation, when the solution is transfused. Even if no gas is lost some remains uncombined, either dissolved in the solution or mixed with the air in the space above. Therefore, if this injection is sterilised by autoclaving, the following precautions must be taken to ensure that the reaction has completely reversed before the preparation is used:

- (i) The injection must be saturated with carbon dioxide before sterilisation, to encourage the back reaction.
- (ii) A gas-tight container, e.g. a transfusion bottle, must be used, to prevent loss of carbon dioxide, especially during sterilisation.
- (iii) The container must not be opened for at least 2 hours after cooling to room temperature, to allow time for complete reformation of bicarbonate.

##### (c) Mersalyl Injection

Mersalyl is the sodium salt of a complex organic acid containing mercury. This complex decomposes in solution, especially in the presence of salts, and liberates toxic mercuric ions. The sodium chloride in tissue fluids would induce this breakdown if mersalyl alone was injected.

Mersalyl combines with compounds that contain an acidic nitrogen atom, e.g. theophylline, the linkage taking place at the mercury atom and making its liberation more difficult.

It is deliquescent and needs protection from light and, therefore, is made from the more stable, but sparingly soluble, mersalyl acid during the preparation of the injection. The acid and the stabiliser, theophylline, are suspended in water and sodium hydroxide is added until solution has been effected. Then the pH is adjusted to the optimum for stability.

(For a review of the stabilisation of injections see Grainger and Carr, 1955.)

is tilted head downwards. Careful choice must be made of the specific gravity of the solution and the position of the patient so that the movement of the injection will be in the desired direction. Although the specific gravity of the cerebrospinal fluid is not constant an average figure is 1.0059 at 37°C. The terms used to describe the specific gravity of injection solutions in relation to that of the spinal

fluid are isobaric, hypobaric and hyperbaric, i.e. of equal, lower and higher specific gravity respectively. For example, the following solutions are used.

Cinchocaine hydrochloride, 1 in 1,500, in 0.5

per cent saline. This is a hypobaric solution and has a specific gravity of 1.0036 at 37°C.

Cinchocaine hydrochloride, 1 in 200, in 6 per cent dextrose. This is a hyperbaric solution and has a specific gravity of 1.02.

## PRESENTATION OF SUSPENSIONS

Inevitably, interest in depot therapy has resulted in an increase in the number of injections that contain the medicament in suspension. This development has presented a surprising number of problems in formulation and resulted in some exceptionally interesting preparations.

The chief difficulty of dispensing a drug in suspension is to ensure accurate dosage, especially if multiple-dose containers are used. This cannot often be overcome simply by using the drug in a finely divided condition (e.g. 120 powder) so that after following directions to shake well it remains suspended long enough for removal of the correct dose. The following factors may interfere:

### 1 Wettability

Some substances, e.g. cortisone acetate, hydrocortisone acetate and procaine benzylpenicillin, are not only insoluble or almost insoluble in water but are also poorly wetted by it. Consequently, if attempts are made to prepare simple aqueous suspensions it is difficult to break up clumps, and the foam produced by shaking takes a long time to disperse because it is stabilised by the film of unwettable powder at the liquid/air interfaces. To ensure that the solid is satisfactorily wetted it is necessary to reduce the interfacial energy between it and the liquid. This can be done by adding a suitable wetting agent and non-ionic types are widely used e.g. the polyoxyethylene ethers of the fatty acid esters of the sorbitans (known in America as the Tweens).

### 2 Sedimentation Rate

Even if a clump-free dispersion can be made, with or without a wetting agent, the preparation will be difficult to use if the particles sediment very quickly. To counteract this a hydrophilic colloid can be added to increase the viscosity of the vehicle, which then holds the particles in suspension long enough for an accurate dose to be removed, at the same time it helps to prevent the reformation of clumps.

A desirable property of a suitable colloid is rapid solution because suspensions often have to be made just before use. One of the most popular choices is sodium carboxy methylcellulose.

### 3 'Claying'

A suspension in which all the solid has been broken down into individual particles (a condition known as deflocculation) is often unsatisfactory because when these small particles settle they produce a very tightly packed sediment that is hard to disperse and is said to have 'clayed'. This can be avoided by reducing the amount of wetting agent, sufficient is added to take the powder into suspension but the quantity is not enough to break down the smaller agglomerates. Such a suspension (referred to as partially flocculated) will settle more quickly but the sediment is loose and easy to redisperse.

To summarise, elegant suspensions of poorly wettable substances can be made by adding a wetting agent but the amount is controlled to prevent complete deflocculation which might lead to claying. Too rapid sedimentation is prevented by including a hydrophilic colloid.

## OTHER FEATURES OF SUSPENSION FORMULATION

### 1 Size, Shape and Stability of Particles

The size of the particles will influence the depot effect of a suspension, larger particles producing the greatest prolongation because they take longer to be absorbed. This is well illustrated by crystalline insulin-zinc suspensions, where large crystals cause the most protracted hypoglycaemic effect.

However, large particles sediment quickly, cause more pain on injection and tend to block syringe needles and, therefore, it may be necessary to exclude, or limit, very large sizes.

Approximately isodiametric crystals, e.g. cubes (insulin) and platelets (procaine penicillin), are better than long needles because the latter are much more likely to cause needle blockage.

In certain cases, e.g. cortisone acetate, care must be taken to prepare the crystals by a method that will produce a stable structure (Edkins, 1958). Some crystals are very unstable (Callow and Kennard, 1961) and on storage in suspension, change into more stable forms which, for example, may be too large and lead to the difficulties mentioned above.

## 2 Thixotropy

Although claying can be prevented by flocculating a suspension it is possible to overdo this and produce a preparation in which the particles are so highly flocculated that, if the concentration of solids is fairly high, the preparation becomes an unpourable paste. However, if the particle sizes and percentages of powder are carefully chosen these pastes are thixotropic, i.e. they are solid in the absence of a shearing force but become fluid if tapped or shaken, the original structure being resumed after a few minutes at rest. Thixotropic preparations have the advantage that the particles remain in more or less permanent suspension during storage and yet, when required for use, the pastes are readily made fluid by tapping or shaking. The shearing force on the injection as it is pushed through the needle ensures that it is fluid when injected but the rapid resumption of the gel structure prevents excessive spreading in the tissues and, consequently, a more compact depot is produced than with non-thixotropic suspensions. Aqueous procaine penicillin injections have been formulated in this way, especially in America (see Ober, Vincent, Simon and Frederick, 1958).

## SUSPENSIONS IN OILY VEHICLES

As depot preparations, oily suspensions have the potential advantage of combining the retarding effects of a hydrophobic vehicle and an insoluble medicament. However, because of the disadvantages of oily vehicles (q.v.) aqueous formulations are preferred by the medical profession even though they may not have quite as powerful a depot effect. The injections of procaine penicillin illustrate this well (see Eastland (1951) and Woodard (1952)), the original depot forms were suspensions in oil but, in this country at least, these have been almost entirely displaced by the more convenient aqueous suspensions. The *United States Pharmacopoeia* contains aqueous and oily procaine penicillin suspensions but the *British Pharmacopoeia* contains only the aqueous type. Nevertheless, the oily preparations illustrate developments in formulation that are of great importance.

### 1 Addition of a Gelling Agent

In the oily procaine penicillin injection of the *United States Pharmacopoeia* the medicament is suspended in peanut (arachis) or sesame oil that has been gelled with 2 per cent of aluminium monostearate. The latter produces a molecular latticework that gives rigidity to the preparation. However, the gel is thixotropic and can be broken down for injection by the methods mentioned for aqueous thixotropic

suspensions. Procaine penicillin in oil has a great tendency to clay if allowed to sediment, the rigid stearate gel prevents this.

### 2 Particle Size

Crystalline insulin zinc has been used above to illustrate that in a simple suspension, as would be expected, larger particles give a greater depot effect. However, the opposite is the case for preparations of procaine penicillin in oil containing aluminium stearate, i.e. reduction of particle size gives increased prolongation. Aluminium stearate is water-repellent and if it is assumed (there is no definite proof) that the particles of procaine penicillin are coated with it they will be less easily reached by the aqueous tissue fluids, and absorption will be further delayed. The greater the subdivision of a powder, the greater its surface area and, consequently, the greater the area of protective coating i.e. in a suspension of fine particles more of the drug is covered with repellent stearate and, therefore, a longer depot effect is produced than with a suspension of large particles.

The very fine particles required for this type of suspension (majority under 5 microns) are produced by air reduction mills ('micronised' powders). The broader particle size distributions necessary for satisfactory thixotropic aqueous preparations are obtained by mixing 'micronised' powders with smaller amounts of 'milled' powders obtained from hammer or ball mills, these produce particles that are about 10 times larger than the micronised particles.

(For further information on suspension formulation see Frederick (1961), Jack (1959), Martin (1961), Oldshue (1961) and Samyn (1961)).

## REFERENCES

- 1 AMERICAN MEDICAL ASS (1954) Report of the council on pharmacy and chemistry of the American Medical Association *J Amer med Ass* 156, 1582
- 2 BACTERIOSTATIC SUB-COMMITTEE REPORT (1954) Conference on the control of antibiotics Stability of Penicillin solutions *Pharm J* 172, 229 and 231
- 3 B.D.H. (1961) *pH values* 7th Ed British Drug Houses Ltd., Poole
- 4 BULLOCK, K. (1938) The preparation of alkaline buffered solutions of procaine hydrochloride for surgical use *Quart J Pharm* 11, 407
- 5 BULLOCK, K. and CANVELL, J. S. (1941) The preparation of solutions of procaine and adrenaline hydrochlorides for surgical use *Quart J Pharm* 14, 241-251

- 6 CALDER, G (1958) Purification of water by ion-exchange resins *Public Pharmacist* 15, 96-101
- 7 CALLOW, R. K and KENNARD, O (1961) Polymorphism of cortisone acetate *J Pharm Pharmacol* 13, 723-733
- 8 CARPENTER, C. P and SHAFFER, C. B (1952) Polyethylene glycols as injection vehicles *J Amer pharm Ass, Sci Ed* 41, 27-29
- 9 CARR, T and WING, W T (1951) The stabilisation of solutions of benzylpenicillin. *Pharm J* 167, 63-65
- 10 CHAKRAVARTY, D C and JONES, J W (1957) A study of solubilising agents in the preparation of stable calcium gluconate solutions for parenteral use *Drug Standards* 25, 4-10
- 11 CIBA (1955) *Nupercaine Handbook* 3rd. Ed. Ciba Labs Ltd Horsham.
- 12 CLAPHAM, P C (1950) The stability of penicillin *Pharm J* 165, 126-129
- 13 COOK A M and SAUNDERS, L (1962) Water for Injection by Ion Exchange *J Pharm Pharmacol* 14, 83T-86T
- 14 COOPER, J W and GUNN, C (1957) *Tutorial Pharmacy* 5th Ed. Pitman Medical London
- 15 COOPER, J W and GUNN, C (1957a) ibid 189-193
- 16 COOPER, J W and GUNN, C (1957b) ibid 565-568
- 17 COOPER, J W and GUNN, C (1957c) ibid 157-160
- 18 COOPER J W and GUNN, C (1957d) ibid 226-230
- 19 DAVIS, H. (1948) Preservatives in solutions for parenteral use *Quart J Pharm* 21, 451-454
- 20 DAVISSON, E O et al (1956) Preservation of poliomyelitis vaccine with stabilised thiomersal *J Lab clin Med* 47, 8
- 21 DYKES, P W (1962) Fatal pyrogenic reactions in man *Lancet* i, 563-564
- 22 EASTLAND, C J (1951) Some aspects of modern formulation *J Pharm Pharmacol* 3, 942-959
- 23 EDKINS, R P (1958) Recent trends in formulation *Pharm J* 180, 97-99
- 24 EISMAN, P C, JACONIA, D and MAYER, R. L. (1953) The preservation of parenteral vegetable oils by chemical agents *J Amer pharm Ass Sci Ed* 42, 659-662
- 25 FREDERICK K J (1961) Performance and problems of pharmaceutical suspensions *J Pharm Sci* 50, 531-535
- 26 FROST, A E (1956) Applications of chelating agents *Mfg Chem* 27, 412-413
- 27 GERSHENFELD, L. (1952) Benzyl alcohol in parenteral solutions *Amer J Pharm* 124, 399-404
- 28 GRAINGER H S and CARR, T (1955) Stabilisation of injectable solutions *Indian Pharm* 10, 319-327
- 29 HADGRAFT, J, HOPPER, C G and SHORT, R (1951) Stability of aqueous solutions of crystalline penicillin sodium. *Pharm J* 167, 13-15
- 30 HARTMAN, C W and HUSA, W J (1957) Isotonic solutions, V The permeability of red corpuscles to various salts *J Amer pharm Ass, Sci Ed* 46, 430-433
- 31 HARTSHORN, E A (1953) A review of preservatives used in parenterals *Amer J Pharm* 125, 365-387
- 32 HOLT, L B (1944) Preservation of parenteral solutions *Mfg Chem* 15, 349-352
- 33 HUSA, W J and ADAMS, J R. (1944) Isotonic solutions II The permeability of red corpuscles to various substances *J Amer pharm Ass, Sci Ed* 33, 329
- 34 INGOLD, K U (1961) Inhibition of the autoxidation of substances in the liquid phase *Chem Rev* 61, 563-589
- 35 INTERNATIONAL PHARMACOPOEIA (1955) Vol II 1st Ed World Health Organisation, Geneva p 106
- 36 JACK, D (1959) Pharmaceutical dispersions of solids in liquids *Mfg Chem* 30, 151-153
- 37 LABRATIS Technical literature from Jacobson Van der Berg and Co Ltd London, E C 3
- 38 LUND C G, PEDERSEN BJERGAARD, K. and BECKER RASMUSSEN E (1950) The preparation of solutions iso-osmotic with blood, tears and tissues II *Acta Pharm Intern* 1, 3-11
- 39 LUPTON A. W (1942) The pain effects of injections of varying pH *Pharm J* 148, 105
- 40 MARCH, B, SOMMERS L O and MOORE, E. E. (1952) Solubilising effects of calcium salts upon calcium gluconate *J Amer pharm Ass, Sci Ed* 41, 366-367
- 41 MARTIN, A N (1961) Physical approach to the formulation of pharmaceutical suspensions *J Pharm Sci* 50, 513-517
- 42 MEDICAL RESEARCH COUNCIL (1962) *The sterilisation use and care of syringes* M R C Memor No 41 H M Stationery Office London.
- 43 OBER, S S, VINCENT, H C, SIMON D E and FREDERICK K J (1958) A rheological study of procaine penicillin G depot preparations *J Amer pharm Ass Sci Ed* 47, 667-676
- 44 OLDSHUE, J Y (1961) Mixing of solid liquid suspensions *J Pharm Sci* 50, 523-530
- 45 PERKINS, J J (1956) *Principles and methods of sterilisation* Charles C Thomas Springfield, Illinois
- 46 PLATCOW, E L and VOSS, E (1954) Isopropyl myristate as a vehicle for parenteral injections *J Amer pharm Ass, Sci Ed* 43, 690-692

- 47 POCKET BOOK (1960) *Pharmaceutical Pocket Book*  
17th Ed The Pharmaceutical Press, London  
178-182
- 48 ROBINSON, F A and PAGE, J E (1942) The stabilisation of stibophen solutions *Quart J Pharm* 14, 40-44
- 49 ROSCOE, C W and HALL, N A (1956) Chelating agents as stabilisers for epinephrine hydro chloride *J Amer pharm Ass, Sci Ed* 45, 464-470
- 50 SABATINI, G R, GULESICH, J J and DOERGE, R F (1956) The pharmacy of chlorpromazine *J Amer pharm Ass, Practical Pharmacy Ed* 17, 454-457
- 51 SAMYN, J C (1961) An industrial approach to suspension formulation *J Pharm Sci* 50, 517-522
- 52 SAUNDERS, L and SHOTTON, E (1956) Water for pharmaceutical purposes *J Pharm Pharmacol* 8, 832 839
- 53 SCHROETER, L C (1961) Sulphurous acid salts as pharmaceutical preservatives *J Pharm Sci* 50, 891-901
- 54 SENGUPTA, S G and ROY, B G (1949) Studies in the supersaturation of calcium gluconate solution *Pharm J* 163, 163-4
- 55 SHOTTON, E and HABEEB, A F S A (1954) The entrainment of liquid during distillation *J Pharm Pharmacol* 6, 1023 1035
- 56 SMITH, R L (1959) *The sequestration of metals* Chapman and Hall, London Chap IX
- 57 SWALLOW, W (1952) Solutions of crystalline penicillin *Pharm J* 168, 467-468
- 58 SWIFT, H B (1950) A note on bactericides in solutions for injection *J Pharm Pharmacol* 2, 101-104
- 59 SYKES, G (1958) The basis for sufficient of a suitable bacteriostatic in injections *J Pharm Pharmacol* 10, 40T-46T
- 60 SYKES, G and HOOPER, M C (1954) Phenol as the preservative in insulin injections *J Pharm Pharmacol* 6, 552-557
- 61 THOMASSON, C L and HUSA, W J (1958) Isotonic solutions, VII The permeability of red corpuscles to various alkaloidal salts *J Amer pharm Ass, Sci Ed* 47, 711-714
- 62 TODD, J P (1955) Bacterial pyrogens *J Pharm Pharmacol* 7, 625-641
- 63 WEST, G B (1945) Solutions of adrenaline *Pharm J* 155, 86-87
- 64 WEST, G B (1947) The stability of adrenaline solutions Part IV Solutions of Adrenaline ascorbate and other storage experiments *Quart J Pharm* 20, 541-545
- 65 WHITTET, T D (1954) The occurrence and importance of pyrogens *J Pharm Pharmacol* 6, 304-309
- 66 WHITTET, T D (1956) The action of ion-exchange resins on pyrogens *J Pharm Pharmacol* 8, 1034-1041
- 67 WHITTET, T D (1959) Methods of purifying water for pharmaceutical purposes *Public Pharmacist* 16, 33-40
- 68 WHITTET, T D (1961) Deionising plants and the pyrogenicity of London tap water *Pharm J* 187, 129-131
- 69 WOODARD, W A (1952) Recent developments in the pharmacy of antibiotics *J Pharm Pharmacol* 4, 1009-1046
- 70 YEH, S Y and LACH, J L (1961) Stability of morphine in aqueous solutions, III *J Pharm Sci* 50, 35-42

# 21

## Containers and Closures

### CONTAINERS

#### DESIRABLE FEATURES OF AN INJECTION CONTAINER

- 1 The material from which it is made should not affect the contents, some types of glass yield appreciable amounts of alkali or shed flakes, while plasticisers, catalysts and other adjuncts may be dissolved from certain plastics.
- 2 The container must be strong enough to withstand the changes of temperature and pressure associated with heat sterilisation methods.
- 3 It should protect the contents from harmful light radiations
- 4 In hospital practice, for economic reasons, it must be suitable for repeated use and, consequently, it should be easy to clean. Alternatively, it must be cheap enough to discard when empty.
- 5 To allow critical examination of the contents for particles and signs of decomposition it should be transparent and colourless. It should keep its transparency for several years, under a variety of climatic conditions, in contact with the many different solutions used in injections, and in spite of repeated steam sterilisations.

### I. GLASS

Glass is the only material that has been used to a significant extent for the containers (as distinct from the closures) of injections and although some specialised applications in this field have been found for plastics, the glass container is unlikely to be seriously challenged in the immediate future. Therefore, the pharmacist must be fully aware of the problems of packing injections in glass.

#### A ALKALINITY

##### 1. Source

Container glass is made by fusing, at very high temperatures, silica (in the form of sand) with two or more other materials, e.g. soda ash and limestone.

Glass made from pure silica consists of a three dimensional network of silicon atoms each of which is surrounded by four oxygen atoms in the form of a regular tetrahedron (Fig. 21.1). Every oxygen atom is joined to two silicon atoms and in this way the tetrahedra are linked together to produce the net work. To illustrate the discussion that follows it is

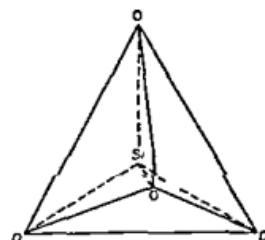


Fig. 21.1 SILICA TETRAHEDRON

convenient to represent this solid structure by a formula in one plane (Fig. 21.2).

Articles of fused silica are very resistant to chemical attack, thermal shock and the risks of handling but, because of the high melting point of this material, they are extremely difficult to manufacture and, consequently, very expensive. Glasses of lower

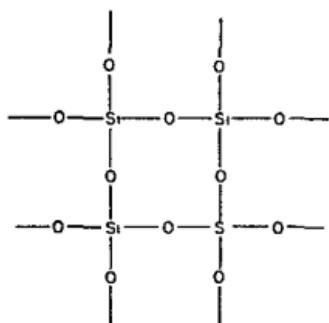


Fig. 21.2 SILICA NETWORK

Represented in one plane

melting point, that can be processed and manipulated much more easily, are produced by the inclusion of other substances, as follows—

**ALKALINE OXIDES** (e.g.  $\text{Na}_2\text{O}$ , added in the form of soda ash (crude sodium carbonate))

These break up the network by splitting some of the silicon oxygen bonds (Fig. 21.3). Because the resulting structure is less rigid it has a lower melting point and is easier to mould, but the replacement of the strong covalent silicon oxygen bonds by the relatively weak, electrovalent alkali oxygen linkages produces a glass that is mechanically and chemically much less durable. This may be more easily appreciated if this type of glass is pictured as fused silica in which some of the insoluble silica has been replaced by water soluble sodium silicate. In fact, satisfactory glass cannot be made from silica and alkali oxide only, the product is too rapidly attacked by aqueous solutions and atmospheric carbon dioxide (see weathering)

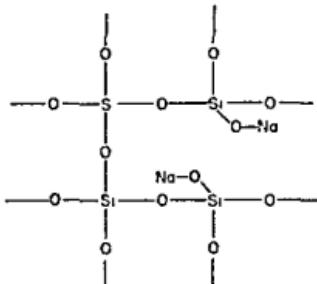
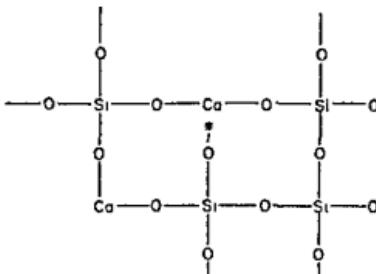


Fig. 21.3 SILICA-ALKALINE OXIDE NETWORK

**OXIDES OF DIVALENT ELEMENTS** (e.g.  $\text{CaO}$  (often used as limestone),  $\text{CaCO}_3$ ,  $\text{BaO}$ ,  $\text{MgO}$ ,  $\text{PbO}$ , and  $\text{ZnO}$ )

Unlike the alkaline oxides, these do not actually break the network, they merely push the tetrahedra apart (Fig. 21.4). This bonding produces a stronger structure than that obtained with alkaline oxides and the chemical resistance is accordingly improved.

The most widely used type of glass contains both calcium and sodium oxides, and is known as lime-soda. The sodium oxide makes the material easy to process and mould while the calcium oxide produces a degree of chemical resistance that is adequate for many types of container, e.g. medicine bottles.



\* Connected to another calcium ion or tetrahedron

Fig. 21.4 SILICA-ALKALINE EARTH OXIDE NETWORK

**BORIC AND ALUMINIUM OXIDES** ( $\text{B}_2\text{O}_3$ ,  $\text{Al}_2\text{O}_3$ )

Because of the relatively low chemical durability, heat resistance and mechanical strength of lime-soda glasses, compared with fused silica, attempts were made to increase the flexibility of the silica network (and, consequently, the ease of moulding of the glass) without the use of large amounts of sodium and calcium oxide. Considerable success was achieved by the use of boric and, to a lesser extent, aluminium oxide. Since boric oxide, like silica, is acidic, it does not disrupt the network but forms tetrahedra itself; however, these are not the same size as the silicon tetrahedra with the result that the lattice becomes distorted, and this produces flexibility. Aluminium oxide, which is amphoteric, can behave similarly. These glasses have the advantages of fused silica although not to the same degree.

(For further information on the structure of glass see Dimbleby (1953), Greene (1961), Loewenstein (1948) and Stevels (1960/61))

## 2. Effects of Alkalinity

Glass containers very easily yield alkali ions to aqueous preparations. Because many injections are stored for long periods, during which alkali extraction could be considerable, and a number of their medicaments are unstable at high pHs, the *British Pharmacopœia* requires the containers used for alkali sensitive substances to comply with a limit test for alkalinity. The importance of this requirement is demonstrated by the following examples of effects produced by high alkalinity.

### (a) PRECIPITATION

#### *Alkaloids*

A number of injections contain alkaloidal salts, e.g. Injections of Apomorphine (hydrochloride), Atropine (sulphate), Emetine (hydrochloride), Ergometrine (maleate), Ergotamine (tartrate), Hyoscine (hydrobromide), Methylergometrine (maleate) and Morphine (sulphate). These salts are decomposed by alkaline substances with liberation of the free alkaloid. Alkaloids, i.e. the bases, are practically insoluble in water and may be precipitated. However, since all alkaloids are slightly water-soluble and the prescribed doses of their salts are often very small (e.g. Hyoscine hydrobromide—up to 0.6 mg in 1 ml) precipitation may not occur although the alkaloid is liberated, consequently, in injections this effect is not as serious as might be expected.

#### *Polypeptides*

The isoelectric point of insulin lies within the pH range 5.5 to 5.6 and, therefore, it will be precipitated if the pH of the injection (officially adjusted to 3.0 to 3.5) is raised to this point by alkali extracted from the container.

### (b) DECREASED STABILITY

#### *Alkaloids*

The instability of alkaloids is indicated by a study of the injections of the *British Pharmacopœia*. Among the methods of stabilisation necessary are pH adjustment (ergot alkaloids, emetine), replacement of air with nitrogen (apomorphine, ergot alkaloids and tubocurarine) and inclusion of sodium metabisulphite (apomorphine and morphine sulphate). For morphine sulphate and emetine the low-temperature method of sterilisation, Heating with a Bactericide, must be used and, in most cases, protection from light is directed. Generally, the instability of these substances is increased by rise of pH and, therefore, the containers must comply with the test for alkalinity, e.g.—

*Apomorphine* The hydrochloride of this alkaloid is official and, if stabilised by the use of Water for Injection free from Dissolved Air, sodium metabisulphite and nitrogen filling can be sterilised by autoclaving. The sterilised solution may be stored indefinitely if protected from light. However, apomorphine itself is very rapidly oxidised to a green compound and, therefore, if a solution of the hydrochloride is sterilised or stored in a container yielding more than a trace of alkali the solution will turn green.

*Atropine* The pH range most favourable for maximum stability of atropine is approximately 3.0 to 4.0. Above 4.5 the decomposition (which is by hydrolysis, atropine being an ester) is catalysed by hydroxyl ions. Consequently, storage in high alkali glass will speed up its destruction and 90 per cent loss has been reported in an 0.1 per cent aqueous solution for which lime-soda glass was used as the container (see Kondzitzer and Zvirblis, 1957).

*Morphine* Solutions of morphine sulphate develop colour on heating and during storage, this is retarded by acid and accelerated by alkaline pHs. Consequently, morphine solutions must be packed in low alkali glass. The use of metabisulphite in the official injections avoids the use of the very low and painful pH (approximately 3) that would otherwise be necessary (see Foster, MacDonal and Whittet (1950) and Yeh and Lach (1961)).

#### *Polypeptides*

*Insulin* This complex polypeptide is most stable between pH 2.0 and 4.0; it rapidly decomposes when the reaction is above 7.0. Therefore, protection from alkali is necessary.

*Posterior Pituitary Hormones* These are represented in the *British Pharmacopœia* by the injections of oxytocin (the oxytoxic hormone) and vasopressin (the pressor hormone). For maximum stability both must be adjusted to within the pH range 3.0 to 4.0. Work by Wokes (1932) on Posterior Pituitary Injection (now in the *British Pharmaceutical Codex*, it contains both active principles but has been replaced in the B.P. by separate injections of the purified hormones) showed that at pH 3 to 4 it lost 10 to 20 per cent of the oxytoxic activity during sterilisation at 115°C for 30 minutes but, if the pH was 5, about 50 per cent was destroyed. Although the official injections are no longer sterilised by a heating method, alteration of pH in either direction accelerates decomposition on storage (Nielsen, 1959) and, therefore, low alkali containers are necessary.

### Synthetic Bases

*Adrenalin* This substance decomposes very easily (see p 391) and although the injection of the *British Pharmacopœia* is of satisfactory stability it must be protected from alkali because even a slight decrease in its acidity causes a significant loss of potency during sterilisation.

*Procaine* The instability of alkaline solutions of procaine was referred to on p 266. Aqueous solutions of procaine hydrochloride are most stable at pH 3.3 and the loss on autoclaving and subsequent storage is not significant if the acidity is less than 4. At alkaline pHs rapid hydrolysis to diethylaminoethyl alcohol and para aminobenzoic acid occurs. The most important official preparation is Procaine and Adrenaline Injection B.P. (pH about 4) which is used as a local anaesthetic in dentistry and minor surgery, the adrenaline is used to prolong the anaesthesia. Since both of the active ingredients need protection from alkali it is essential to use low alkali containers.

### Vitamins

The stability of many vitamins (e.g. aneurine, ascorbic acid and cyanocobalamin) is greatly reduced in alkaline solutions and the use of containers of low alkalinity is an important means of preserving their activity (see Heathcote and Wills, 1962).

The above are only a few of the many injections to which the Pharmacopœial requirement applies. The complex chemical structure of many modern medicaments increases the likelihood of instability, and protection from alkali is often necessary (e.g. Tubocurarine, Neostigmine, Mephenesin and Picrotoxin Injections). In most cases where, to improve stability, a pH adjustment is made (e.g. Bismuth Sodium Tartrate, Heparin and Mersalyl Injections) or a buffer is included or suggested (e.g. Leptazol, Stibophen and Suxamethonium Chloride Injections) low-alkali glass is required in addition.

### (c) INFLUENCE ON STERILITY

Since pHs of below 4 are bactericidal it might seem unnecessary to include a bactericide in multi dose containers of very acid injections (e.g. Insulin and Globin Insulin, both 3.0 to 3.5, Nalorphine, 2.7 to 3.3, Oxytocin and Vasopressin, both 3.0 to 4.0, and Adrenaline, 3.2 to 3.6). However, the B.P. makes no exceptions in these cases and one of the reasons is that glass, even if it complies with the limit test, may liberate sufficient alkali during prolonged storage to raise the pH above 4. If this happened the injection would no longer kill and might not even inhibit bacteria.

### 3. Glass for Injection Solids

In the *British Pharmacopœia* there are medicaments which are so unstable in solution that it is necessary to prepare the injection *immediately* before use. Examples are amethocaine hydrochloride, chorionic gonadotrophin and tryparsamide. A sterile solvent is added aseptically (i.e. in a manner that prevents bacterial contamination) to the sterile medicament in its sterile container (which is usually an ampoule) when required. Since alkali extraction is not a problem during the storage of a dry drug in a well-closed container and, after the injection has been made, the solution is not in contact with the glass long enough for significant extraction to occur, the B.P. does not specify the use of low-alkalinity glass for such substances.

Where, however, the same method of preparation is used but the injection is sufficiently stable to be stored, under suitable conditions, for several days (e.g. the penicillin and streptomycin injections) alkali extraction must be anticipated and satisfactory glass used.

### B LOSS OF BRILLIANCE

In a damp atmosphere moisture condenses on the surfaces of glass containers and extracts some of the weakly-bonded alkali ions from the network. When the surfaces become dry, a white deposit is left which consists mainly of alkali carbonate produced by interaction of the alkali with carbon dioxide from the air. If this film is washed off with water or weak acid the exposed glass surface will contain considerably less alkali and, therefore, subsequent extraction will be at a slower rate. However, if the film is allowed to remain, when further condensation occurs an alkaline solution is produced at once and this dissolves away some of the silica (because of the ease with which alkalis can disrupt the network) with a resulting loss of surface brilliance. This is known as 'weathering'.

Loss of brilliance can be caused in ways other than weathering. When water is kept in high-alkali containers the extracted alkali is replaced by hydrogen ions to maintain the electrical balance. These are accompanied by some water molecules which cause the network near the surface to swell slightly. When the container is emptied and dried the loss of this water leads to cracks in the surface.

Apart from the aesthetic objection to containers that have lost their brilliance, they are quite unsuitable for injections because it is essential to see easily the earliest signs of decomposition such as faint precipitates and slight colour changes.

The problem is most acute for the hospital pharmacist who has to re-use most of his containers. For small volumes the higher cost of low alkalinity (neutral) glass may not be prohibitive and, for large volumes, bottles that have been surface-treated to reduce alkali extraction can sometimes be obtained. But, if it is necessary to use some containers of quite high alkalinity they should be thoroughly and quickly dried after cleaning and stored in a dry, well ventilated place.

### C FLAKING

Mention has been made of the silica rich layer produced on the surface of glass containers as a result of alkali extraction. Sometimes, parts of this layer fall away and can be seen as glistening flakes in the contents. Separation may be due to the different chemical compositions and, therefore, rates of expansion of the silica rich layer and the rest of the glass below.

Flakes are produced most readily by alkaline solutions because these roughen the surface by eating away parts of the silica. Consequently, flakes appear quite quickly when injections containing sodium citrate or phosphate are packed in certain types of glass. Occasionally, and more slowly, they develop in injections that contain sodium, potassium and calcium chlorides, and because solutions of these salts are often given intravenously in large volumes the presence of insoluble matter, particularly glass, is most undesirable. Therefore, the *British Pharmacopoeia* gives a warning in the monographs of Sodium Chloride, Sodium Chloride and Dextrose, Sodium Lactate and Compound Sodium Lactate Injections that small particles may separate from a glass container, and it also forbids the use of solutions containing such particles.

A particularly serious problem is presented by human blood, in which citrate is used as an anti-coagulant and flaking is very hard to detect because of the colour and the presence of the cells. The production of an acid pH by the use of sodium acid citrate has considerably reduced the hazard but it is still necessary to choose the glass for blood bottles carefully.

Although flaking is less common in low-alkali glasses they are not immune. Therefore, it is desirable to test samples for suitability before bringing a new type of container into use on a large scale. Surface treatments (q.v.) are only a partial solution to the flaking problem.

Since flaking is increased by high temperatures it is helpful to store injections in a cool place, to use the minimum effective sterilisation times and to cool rapidly afterwards.

The possibility of flakes in small containers, including ampoules, should not be overlooked.

### D THERMAL RESISTANCE AND MECHANICAL STRENGTH

All glass containers must be strong enough to withstand the risks of normal handling and transport but those used for injections sterilised by heating methods, including steam under pressure, must also resist the additional hazards of sharp changes in temperature and pressure. For example, at the end of sterilisation by autoclaving the pressure inside the apparatus is allowed to fall to atmospheric and then the lid is removed or the door opened. The resulting sudden contact of cool air from the room, with the very hot contents which cool very slowly because of the poor thermal conductivity of their glass walls, may cause breakages which, due to the high pressure inside the closed containers, can occur with explosive violence.

Accidents of this kind can be reduced by the use of glass that has high resistance to thermal shock. Glasses similar to those used for ovenware, in which a large amount of alkaline oxide is replaced by boric oxide, are desirable but, for large containers, expense may prohibit the choice of the best compositions. Reduction of glass thickness is helpful (cf. chemical glassware) but although this is practicable for ampoules it cannot be applied widely to large containers such as half or one litre transfusion bottles because these must have considerable mechanical strength to withstand frequent handling and cleaning.

Even if good quality containers are available, care should always be taken to minimise temperature and pressure changes during heat sterilisation. The full sterilisation pressure should never be suddenly released from an autoclave, the contents of an oven or autoclave should be allowed to cool for as long as possible before the apparatus is opened, and a hot bottle from an autoclave must never be subjected to the shock of a stream of cold water from the tap in an attempt to cool it quickly.

### E TYPES OF GLASS

#### 1. Lime-soda Glass

Ordinary (lime-soda) glass, as used for medicine bottles and many other commercial purposes, contains approximately 75 per cent  $\text{SiO}_2$ , 15 per cent  $\text{Na}_2\text{O}$  and 10 per cent  $\text{CaO}$ , often with small amounts (less than 1 per cent of each) of  $\text{K}_2\text{O}$ ,  $\text{MgO}$  and  $\text{Al}_2\text{O}_3$ . The aluminium oxide improves mechanical strength and chemical durability and makes melting easier, the magnesium oxide, when used to replace part of the lime, reduces the temperature required for

manufacture of the glass and widens the temperature range over which the glass can be shaped into containers

This glass can be manufactured at a convenient temperature, is easy to process and, therefore, is inexpensive. Although it is sufficiently resistant to the action of water for ordinary purposes it is unsuitable as a container material for many injections because

- (a) It yields an appreciable quantity of alkali to water
- (b) Flakes separate comparatively easily
- (c) On repeated use its surface loses some of its brilliance
- (d) Its relatively high coefficient of expansion makes it liable to fracture with sudden changes of temperature

## 2 Boro-silicate Glass

The defects of lime soda glass can be largely overcome by decreasing the proportion of alkali (calcium and sodium oxides) and including boric oxide, the latter improves heat resistance and confers great chemical durability. These compositions are called boro-silicate or resistance glass and are used for chemical glassware, ovenware and containers for alkali sensitive preparations. Aluminium oxide is usually present and the silica content is often slightly increased, with advantages that were mentioned earlier. The approximate composition of Pyrex glass, for comparison with the data given for lime soda, is 80 per cent  $\text{SiO}_2$ , 12 per cent  $\text{B}_2\text{O}_3$ , 2 per cent  $\text{Al}_2\text{O}_3$ , 6 per cent  $\text{Na}_2\text{O} + \text{CaO}$  + other oxides. In this country, because of the development of neutral glasses, borosilicate is not widely used for injection containers although some antibiotic vials are made from it.

## 3 Neutral Glass

Boro silicate glasses have two major disadvantages, they are expensive and difficult to melt and mould. In consequence, manufacturers have produced grades of glass (described as neutral) between boro silicate and lime-soda in composition but with suitable characteristics for pharmaceutical purposes. They are softer and more easily manipulated than boro-silicate but have good resistance to autoclaving, weathering and solutions of pH up to about 8, in fact, their resistance to alkalis and alkaline preparations is superior to that of boro-silicate. Small vials (up to 25 ml approximately) are often made from lengths of tubing which must be drawn uniformly, this is difficult with boro silicate but not with neutral. Large transfusion bottles are too costly

to make in borosilicate glass but they can be produced at acceptable prices from neutral glasses. A typical formula is 72–75 per cent  $\text{SiO}_2$ , 7 to 10 per cent  $\text{B}_2\text{O}_3$ , 4 to 6 per cent  $\text{Al}_2\text{O}_3$ , 6 to 8 per cent  $\text{Na}_2\text{O}$ , 0.5 to 2 per cent  $\text{K}_2\text{O}$  and 2 to 4 per cent  $\text{BaO}$

## 4 Neutral Tubing for Ampoules

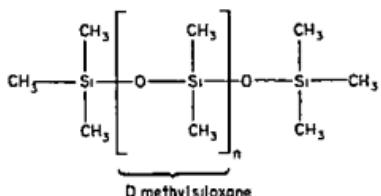
The extensive use of ampoules has made possible the production of special neutral tubing for their manufacture. Its composition differs slightly from the one given above because, after filling, ampoules are sealed by fusion and, therefore, the glass must be easy to melt. Consequently the amounts of alkaline and aluminium oxides are slightly increased and the content of boric oxide and silica slightly reduced, e.g. 67 per cent  $\text{SiO}_2$ , 7.5 per cent  $\text{B}_2\text{O}_3$ , 8.5 per cent  $\text{Al}_2\text{O}_3$ , 8.7 per cent  $\text{Na}_2\text{O}$ , 4 per cent  $\text{K}_2\text{O}$ , 4 per cent  $\text{CaO}$  and 0.3 per cent  $\text{MgO}$ . In spite of these modifications it is generally satisfactory for the storage of alkali sensitive injections. Although the coefficient of expansion is not much less than that of lime soda glass, ampoules made from it have good resistance to thermal shock because of their small capacities and thin walls. Watch should be kept for flaking, which is not unknown.

## 5 Sulphured Containers

An alternative approach in the search for cheaper containers, particularly for large volume injections, is surface treatment. For example, by a process known as 'sulphuring', lime soda glass can be given a neutral surface through which extraction of alkali ions is very small. The containers are exposed to moist sulphur dioxide at above 500°C when the acid gas neutralises the surface alkali to produce a layer of sodium sulphate which can be removed by washing to expose a tough silica rich surface. There is very little published information on how soon ions from the deeper glass penetrate this layer but the fact that the *United States Pharmacopoeia* allows the use of surface treated soda lime glass containers for blood, plasma and the infusion fluids, while the *British Pharmacopoeia* Commission removed from the 1953 edition the crushed glass test for alkalinity, that would not have been applicable to sulphured glass, suggest that experience has been satisfactory. In this country it has been used for dry salt penicillin vials, a safe application because very little alkali will be extracted during the short storage life of the prepared injection.

## 6 Silicone-treated Containers

Silicones are organic compounds containing silicon. They are polymers composed of long chains of



**Fig 21.5 FORMULA OF A SILICONE USED FOR GLASS TREATMENT**

alternating oxygen and silicon atoms with organic groups attached to the latter. The formula of one of the silicones suitable for treating glass is shown in Fig 21.5. By altering the organic groups, the degree of polymerisation, the amount of cross linking between the chains etc., many products are obtained among which are fluids, greases and rubbers. The more solid silicones are a result of a high degree of polymerisation and cross linkage.

Silicones are chemically related to glass and have many of its properties, such as good resistance to heat and oxidation, chemical inertness and freedom from colour, odour and toxicity, but, in addition, the organic groups in silicone molecules confer the valuable property of water repellancy. This characteristic can be given to glass containers by treatment with a suitable silicone. In a typical method the containers are thoroughly cleaned with particular care to remove grease, immersed in a dilute solution of a fluid silicone in an organic solvent, or an emulsion of the silicone in water, drained, heated in a current of air to remove the solvent and, finally, baked to fix the film firmly to the glass. The similarity between the silicon-oxygen bonds of the silicone and the glass probably explains the tight bonding to the glass surface, while an assumption that the organic groups project out from the surface would account for the water repellancy. The siliconed vials available commercially are often treated during manufacture by spraying specially selected silicones into the chamber (lehr) in which the bottles are annealed (i.e. cooled slowly to prevent the development of stresses which would reduce resistance to thermal and mechanical shock).

The advantages of silicone-treated injection containers include

(a) They are not wetted by aqueous solutions or suspensions which, therefore, do not cling to the sides. Consequently, almost the entire contents can be withdrawn by a syringe, and less overage is required.

- (b) With suspensions, e.g. aqueous procaine penicillin, the quick drainage following shaking makes it easy to see the position of the needle in the vial, and the remaining volume.
- (c) Foaming, which can make accurate measurement of the dose difficult, is reduced.

Nevertheless, outside the antibiotic field silicone-coated glassware is not widely used, for the following reasons

- (d) Although the coating certainly makes it more resistant to atmospheric and chemical attack, extraction is not entirely prevented and is increased by repeated sterilisations (Steiger, 1956).
- (e) The film gradually comes away from the glass after frequent autoclaving although, as would be expected from its method of application, it is unaffected by dry heat sterilisation at 150°C (Dumbleby, 1953).
- (f) There is evidence that very little protection is given against flaking (Berry, 1953).
- (g) Occasionally, the coating has an unpleasant greasy appearance. This is very marked with some preparations e.g. Procaine Penicillin with Benzylpenicillin Injection.
- (h) It is very difficult to make normal labels adhere, a special fixative is necessary.
- (i) Chromic acid must not be used for cleaning because it destroys the film.

Most of these disadvantages preclude the use of this treatment for containers that have to be used repeatedly.

(For further information on silicones see Bradley, (1953), Levin (1958), and Thomas (1953, 1954).)

## F TESTS FOR LIMIT OF ALKALINITY

Because none of the glasses used for pharmaceutical containers is completely free from extractable alkali a test for its absence is impracticable and limit tests must be used. In most pharmacopoeias the quantity of alkali yielded to a prescribed solution is estimated and must be less than a specified amount. Ideally, a container should be tested under conditions (e.g. of time and temperature) similar to those it will meet in use, but as this would often make the tests inconveniently long the extraction is accelerated by a high temperature. Two main types of test are used:

### 1 Crushed-glass Test

This is the chief method of the *United States Pharmacopoeia* and until 1953 it was one of the tests of the *British Pharmacopoeia*. The container is crushed and sieved to produce uniform particles of which a

definite weight is taken. Control of the particle size and weight of powder ensures that a constant surface area is exposed to the solution. Because all of the glass (not just the surface layer) is examined and extraction is enhanced by the rough surfaces of the particles, this is a severe test, and if a glass passes it is unlikely that containers made from it will give trouble in use. Nevertheless, the technique is tedious and is not applicable to surface treated containers (sulphured or siliconed) because crushing would expose the alkaline glass below the surface.

## 2. Whole-container Test

This is the method of the *British Pharmacopoeia* and the *International Pharmacopoeia*. It is used in the *United States Pharmacopoeia* for treated soda lime containers only. The containers are simply filled with the test solution and exposed to the test conditions. Because the surface layer of a container is smooth and less reactive than the glass beneath, glassware may pass the whole container test more easily. There can be no serious objection to this, since injection solutions are in contact with the surface and not the deeper layers, but account must be taken of the possible diffusion of alkali from the latter (e.g. as a result of weathering) during the container's lifetime, confirmatory tests will be necessary and acid treatment of the surface (see below) may be required.

Whole-container tests are attractive because they are easier to perform and give more useful information to the practising pharmacist whose primary concern is to discover if the amount of alkali extracted from the surface of the container is sufficient to harm the contents. Nevertheless, the container tests of the three pharmacopoeias mentioned have been criticised because the surface area in contact with unit volume of the solution is not constant for all sizes of container. This is because surface area does not increase as much as volume with increase in container size and consequently, in a test in which the glassware is filled to the nominal volume with attacking solution the smaller sizes are examined more stringently. This point is well illustrated by the following figures from a paper by Steiger (1956).

Inner wetted surface

Container	Volume (ml)	(sq cm)
Ampoule	1	5.9
"	10	29.0
Bottle	1000	511.0

While the millilitre of test solution in a 1-ml ampoule receives the alkali from 5.9 cm<sup>2</sup> of surface, in the 10 ml ampoule and 1 litre bottle each millilitre

gets the alkali from 2.9 and 0.5 cm<sup>2</sup> respectively, i.e. the alkali concentration in the small container will be much greater—about 12 times larger in the 1-ml ampoule than in a litre bottle. However, as these conditions exist when the containers are in use, the contents of small containers being exposed to more alkali than the contents of large containers, this criticism has little practical significance. It would be valid if a manufacturer, finding that a particular glass produced large containers that passed the test, used it for small containers without further check, but this is improbable. It is noteworthy that the *Swiss Pharmacopoeia* determines the alkali release per unit of container surface.

## 3. Official Methods

### (a) THE BRITISH PHARMACOPOEIA, 1963

Six containers must be used and all must pass. Before testing they are cleaned by a prescribed method. The attacking medium is a solution of methyl red in carbon dioxide free water containing a small amount of hydrochloric acid. The containers are filled to their prescribed capacities. Extraction is accelerated by heating in an autoclave at 121°C for half an hour. Ampoules are sealed by fusion of glass, and bottles and vials by a loose fitting closure of an inert material, e.g. copper or silver foil. An aqueous solution of methyl red is pink, in dilute solution, at about pH 4.2 and below, and yellow at about pH 6.3 and above. The quantity of hydrochloric acid is just sufficient to produce the pink colour. The container passes the test if, after autoclaving and cooling, the colour of the solution has not changed to full yellow as shown by comparison with the colour produced by adding 0.1 ml of N/20 sodium hydroxide to 10 ml of the original test solution. Containers of coloured glass are opened if necessary and the contents examined on a well-washed, glazed, white tile.

If possible, the test should be carried out within 14 days of use because containers, previously satisfactory, may not pass after storage (due to weathering). If this happens, the containers may be retested after washing internally with a prescribed solution of acetic acid followed by three washings with purified water. If the containers then comply the batch may be used after similar treatment.

### (b) THE INTERNATIONAL PHARMACOPOEIA (1st Ed Supplement, 1959)

Only a whole container test is described, but this is intended as a rapid test for pharmacists, and the fact that manufacturers may wish to use additional tests is recognised.

The important differences from the B P method are

- The extracting medium is degassed redistilled water
- The extract is titrated with acid and the amount required is limited
- Very complete instructions are given for the autoclaving process. This is for 1 hour and venting, heating up, temperature variation at 121°C and cooling are all clearly defined, the B P simply states 'autoclave at a temperature of 121°C for  $\frac{1}{2}$  an hour, cool and examine'
- Tinfoil is used for covering vials and bottles, this is more easily obtainable than the foils of the B P

(both of which would affect the surface area exposed to extraction) are rinsed away with acetone

- Suitable amounts of dried powder and specially prepared distilled water are autoclaved together at 121°C for  $\frac{1}{2}$  an hour, careful directions being given for the use of the autoclave. A conical flask of resistance glass, covered with a beaker, of similar material, that fits snugly on its rim, is used and both are previously digested in the special water, to remove surface alkali
- The extract is decanted, the powder washed and the alkali in the pooled liquids titrated with 0.02 N sulphuric acid, the result is corrected by a blank experiment without the powder

Table 211

Type	Description	Test used	Limits	
			Size (ml)	Volume of acid to neutralise the extract from 10 G of glass (ml)
1	Highly resistant boro-silicate glass	Crushed glass	All	1.0
2	Treated soda lime glass	Whole container	100 or less	0.7
			Over 100	0.2
3	Soda lime glass	Crushed glass	All	8.5
N P	General purpose Soda lime glass	Crushed glass	All	15.0

- The containers must be sterilised upright. The omission of any reference to this in the B P can cause difficulty because if ampoules fall over and test solution enters their narrow necks it may change colour there (due to the greater area of glass in contact with unit volume of solution) although no change takes place in the body of the container. A mean result is obtainable by shaking to mix the two parts but if the ampoules fail it should be appreciated that they were exposed to a more severe test than if they had been upright

#### (c) THE UNITED STATES PHARMACOPOEIA XVI

A whole container test is included for containers of treated (i.e. sulphured) soda lime glass but the main method is a crushed glass test of which the chief features are

- The containers are crushed in a steel mortar and grains within a specified size range are used. Iron particles, from the mortar, are removed with a magnet and agglomerations of fine particles and fine powder adherent to the grains

The U S P tests are used to classify containers into the four types shown in Table 211

Types 1, 2 and 3 are intended for parenteral preparations and type N P for oral and topical products. The type, or types of glass suitable for each injection are stated in the monograph, e.g.

Type 1 is used for most injections and for water for injection

Types 1 and 2 may be used for infusion fluids blood and plasma. Because these preparations require large volume containers, treated soda lime glass is allowed as a less costly alternative to boro-silicate

Types 1 and 3 may be used for most of the oily injections. Type 3 is permissible because the alkali extraction to the non aqueous vehicle will be negligible

Type 3 is specified for thiopentone sodium. It will be remembered that alkali stabilises this medicament (see Water for Injection free from Carbon Dioxide)

#### (d) THE BRITISH STANDARD SPECIFICATION FOR AMPOULES (B S 795 1961)

This includes a test for alkalinity similar to the one in the B P but the directions are more detailed

Important differences are

- They are sterilised upright
- The duration of the test, including heating up, exposure and cooling to room temperature, must not exceed 60 mins
- The contents of coloured ampoules are examined in a clean 50 ml boro-silicate beaker

#### The Narcotine Hydrochloride Test

Precipitation of an alkaloid from its salt (see 'Effects of Alkalinity') has been used as a limit test for alkalinity. Narcotine hydrochloride is chosen because narcotine is almost insoluble in water. The test is applied by filling the containers with a 0.1 per cent solution of the salt, heating in a boiling water bath and examining for the rate of formation and growth of a precipitate. It is used in Germany but not to any extent elsewhere.

#### G PROTECTION FROM LIGHT

Light causes deterioration of many substances (see Appendix 5). Its effect increases as the wavelength decreases and, therefore, ultraviolet rays (15 to 400 m $\mu$ ) are much more harmful than rays in the visible region (400 to 700 m $\mu$ ). Containers of non-actinic glass (i.e. glass that will not transmit the damaging wavelengths) are available for light-sensitive materials.

Light transmission can be influenced by altering

the chemical composition of glass. This is shown by Fig 216, which has been prepared from transmission curves determined by D. K. Hill of the department of Glass Technology of the University of Sheffield and reported by Dimbleby (1953). His samples were from glasses used in the pharmaceutical industry. The results indicate that—

(a) The colourless (curve 1) and blue (curve 2) glasses transmitted high percentages of the ultra-violet wavelengths.

(b) The yellow green (curve 3) transmitted no ultra-violet, and the amber (curve 4) very little, but the medium green (curve 5) was less satisfactory. The yellow green contained chromium and the medium green contained iron, thus indicating that chemical composition, not colour, determines the efficiency of light absorption. For this reason, not all ambers give as good protection as the example used in this investigation.

(c) Transmission is decreased by increasing the thickness of the glass. Although, at first glance, the amber seems less satisfactory than the yellow-green, this is largely due to the greater thickness of the latter. Confirmation is obtained if wavelength is plotted against the extinction coefficient,  $k$ , per millimetre thickness (Fig 217). Since a high value of  $k$  indicates low transmission it is clear that both amber and yellow green are very satisfactory at 400 m $\mu$ .

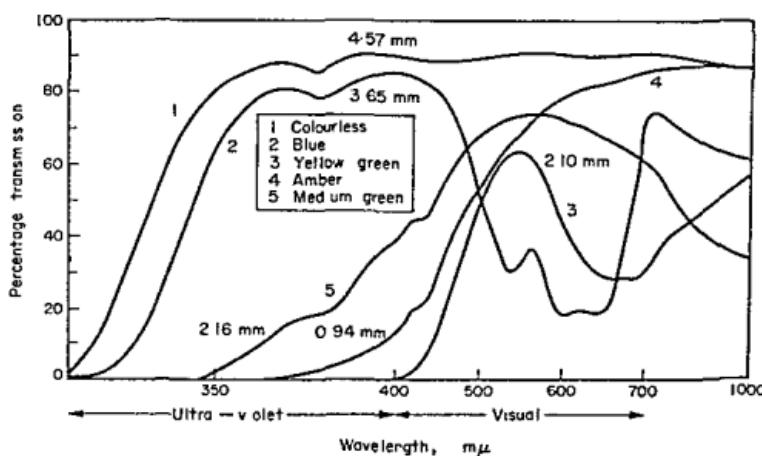


Fig 216 TRANSMISSION CURVES OF CONTAINER GLASSES

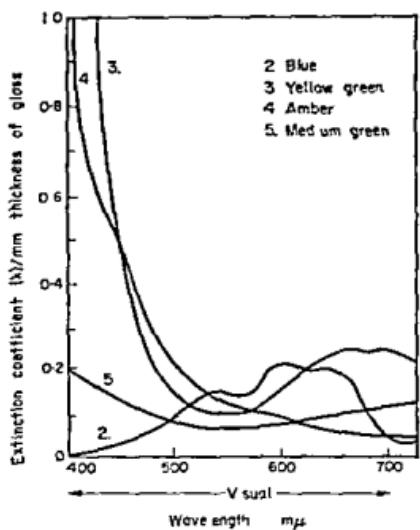


Fig. 217

According to Carlsen (1954, 1957) the exclusion of wavelengths less than 550 mμ reduces the effects of light to an insignificant level, and he suggests that a satisfactory container should not transmit more than 0.1 per cent of wavelengths below this value. To ensure that this is not achieved at the expense of easy visibility of the contents he also recommends that the thickest part of the container should transmit at least 50 per cent of the light of wavelengths above 580 mμ. This wavelength was chosen because it makes the test more stringent, the eye is only about 60 per cent as sensitive to light at 600 mμ as it is at 550 mμ (the wavelength of maximum sensitivity) hence more light is needed to see things clearly.

Containers can be examined for light transmission by using a spectrophotometer, as in the method of the *United States Pharmacopoeia*. In this the average transmission of wavelengths between 290 and 450 mμ is determined and must not be more than 18 per cent.

of the incident light for a container of 2 mm thickness or less. A plane, polished, parallel-surfaced sample of the glass is used. There is no statement about longer wavelengths.

*The British Pharmaceutical Codex* defines a light resistant container as one that does not transmit more than 15 per cent of incident radiation at any wavelength between 300 and 400 mμ.

Carlsen considers that sufficient information is given by the determination of the transmission at a few suitably chosen bands or wavelengths and he recommends using the strong mercury lines at 577 to 579, 546, 436 and 365 mμ. This method has the advantage that a simpler and less costly apparatus than a spectrophotometer can be used, e.g. a suitably adapted Spekker absorptiometer. As standards, he suggests transmissions of more than 75 per cent at 577 to 579 mμ (to ensure good internal visibility) and less than 0.1 per cent at the other wavelengths (to ensure adequate protection). He stresses the need to allow for differences in thickness of the container wall, these are often considerable, particularly between top and bottom.

In practice, many of the containers that give efficient protection from light also appreciably reduce the visibility of the interior. Since priority must be given to the detection of the earliest signs of decomposition, such as slight coloration or a faint precipitate, it is usually more satisfactory to use colourless glass for injections that require protection from light and to pack in a box (for ampoules) or a carton, or light proof wrapping paper such as amber cellulose film (for bottles). Then, a warning label must be added to stress the importance of storing the containers in their boxes or in a dark cupboard. The *British Pharmacopoeia* requires single-dose containers to be made of clear colourless glass unless amber is specifically demanded.

Non actinic glass must be satisfactory in other respects. For example, Girard and Kemy (1950) reported that a type of amber glass liberated sufficient iron to affect the stability of adrenaline hydrochloride solution.

(For further information, see Swartz, Lachman, Urbany and Cooper, 1961.)

## II. PLASTICS

Plastics have improved the packaging of many types of pharmaceutical preparation and the possibility of using them for injection containers, instead of glass, is being constantly investigated. On the whole the materials available at present have disadvantages

that outweigh their advantages but because of the growing and intense interest in this field it is desirable to consider some of the general properties of plastics and the special features of the types that have actual or potential applications in the field of sterile products.

## A GENERAL PROPERTIES OF PLASTICS

- 1 Plastics are synthetic polymers of high molecular weight
- 2 They are sensitive to heat and most of them melt or soften at below 100°C. Consequently, very few are suitable for injection containers that must withstand autoclaving, and only one (polytetrafluoroethylene, PTFE) will withstand dry heat sterilisation
- 3 Light in weight (polythene floats on water) they are easier to handle, making transport cheaper
- 4 Mechanically they are almost as strong as metals and, therefore, containers can have thinner walls than would be needed with glass
- 5 They are poor conductors of heat, a disadvantage if the container and contents have to be heat sterilised. A slight advantage is that plastic containers are not cold to touch
- 6 Generally they are resistant to inorganic chemicals but, apart from PTFE, are often attacked by organic substances, e.g. solvents and oils
- 7 Like rubber, plastics may contain fillers, lubricants, pigments, plasticisers and stabilisers. Unchanged monomer may also be present. Some of these substances can be leached into solutions stored in plastic containers. In addition, chemical bonding between medicaments or preservatives in the solution and chemicals in the plastic may reduce the strength or stability of the former
- 8 Very few types completely prevent the entry of water vapour at all the temperatures to which an injection container may be exposed. Some are permeable to gases, e.g. oxygen and carbon dioxide. Plastics are classified into two groups according to their behaviour when heated

### 1 Thermoplastic Types

On heating these soften to a viscous fluid which hardens again on cooling. Their hardness when cold is influenced by the degree of cross linkage or intermolecular attraction between the long chain molecules. For example, in nylon and high-density polythene considerable cross-linkage produces more rigid materials while polystyrene and polymethyl methacrylate are rather hard and somewhat brittle at room temperature because of strong intermolecular forces linking the molecules.

### 2 Thermosetting Types

When heated, these may become flexible but they do not become fluid, usually their shape is retained right up to the temperature of decomposition. Because of a high degree of cross linking they are usually hard and brittle at room temperature.

## B PLASTICS FOR STERILE CONTAINERS AND EQUIPMENT

### 1. Thermoplastic Types

#### (a) POLYETHYLENE (POLYTHENE)

Polythene is a flexible, very light, but tough plastic that is practically impermeable to water vapour and does not deteriorate with age unless exposed to sunlight for a considerable time. Pure polythene free from plasticisers and other adjuncts, is non toxic to human tissues even when implanted for long periods.

Its most unsatisfactory property is relatively high permeability to gases, penetration of oxygen can lead to deterioration (e.g. discolouration of tetracycline suspensions) and tainting may occur if odorous materials are stored nearby. Other disadvantages are its permeability to certain oils and preservatives, its lack of transparency and the non-adherence of labels. It tends to become charged with static electricity which attracts dust, a problem that is being tackled by the inclusion of antistatics during manufacture or the application of antistatic coatings.

Sterilisation is difficult because its melting point range is 110–115°C and it begins to soften at about 90°C. Cannulae have been sterilised at 104°C for 30 min but they were enclosed in narrow glass tubes to prevent deformation. Similar mechanical support is desirable if steaming or boiling is used, as in the *British Pharmacopoeia* method for the sterilisation of plastic containers (see 'Heating with a Bactericide'). Sterilisation with ethylene oxide gas seems the most satisfactory method.

Because of its water impermeability and ease of fabrication it is widely used for bottles, tubing and heat sealable packaging films.

#### (b) HIGH DENSITY POLYTHENE

This recently developed form of polythene has several important advantages over the low-density material. It is more rigid and, consequently, handling and filling of containers is easier and their walls can be thinner. Its permeability to gases is low and resistance to oils high. Because of its higher melting point it can be sterilised by autoclaving. At present it is expensive but, in America, a type of disposable syringe is made from it.

(For additional general information, see Moxey (1959))

#### (c) POLYVINYL CHLORIDE (PVC)

Compared with normal polythene, PVC is less flexible, heavier and slightly more permeable to water vapour, it is practically unaffected by sunlight and the unplasticised material is non toxic. It is less

permeable to gases than polythene, the surface can be printed readily, and plasticised grades with good oil resistance are obtainable. Some grades can be sterilised by steam under pressure. It has been used for disposable tubing, giving sets and bags for storing blood and infusion fluids. The liberation of acid substances to parenteral solutions by some types of tubing has been reported.

(d) POLYMETHYL METHACRYLATE (P M M A . Perspex)

This is a hard, strong but light, glass-clear material that retains its clarity on exposure. It is used for aseptic screens, heart lung machines and bone replacement. It softens at about 100°C, which precludes the use of a reliable heat sterilisation method.

(e) POLYSTYRENE

Polystyrene is a hard, rigid, light material that is cheap and easy to mould into such items as bottles, tubes, jars, boxes and syringes. These articles produce a unique metallic tinkle when dropped or tapped. It is odourless and tasteless, available transparent or coloured and has excellent dimensional stability that permits the manufacture of components (e.g. caps of tubes, and plungers and barrels of syringes) to fine limits of accuracy. It is rather brittle when cold, and considerably more permeable to water vapour than either polythene or P V C , therefore, moisture sensitive tablets, such as benzylpenicillin, must not be stored in it. With age, and after sterilisation by ethylene oxide, transparent polystyrene may develop fine cracks (crazing), also, it may become yellow on prolonged storage. Like polyethylene, it tends to be electrostatic and it is very permeable to aromatic flavourings.

It softens at a lower temperature than polythene, P V C . or P M M A and because of this and its moisture vapour permeability it is the least suitable plastic for containers of sterile products. Where sterilisation is necessary and the demand justifies it (e.g. disposable syringes), gamma radiation can be used. A well-known application of polystyrene is the plastic measure supplied with oral antibiotic suspensions.

(f) POLYTETRAFLUOROETHYLENE (P T F E )

This is a translucent or opaque material rather like polythene in appearance and mechanical properties but possessing excellent heat resistance, it is unchanged at 250°C. It resists all known solvents and chemicals except gaseous fluorine and molten alkali metals. Because its surface is wax-like and hydrophobic its water absorption is nil and its moisture vapour permeability is even less than that of polythene. It is very expensive and difficult to fabricate.

Although, so far, it has not been used widely in the field of sterile products (but see p. 490) its properties suggest that applications may be found in the future.

(For further information, see Fergusson (1948) and Harris (1962))

(g) POLYPROPYLENE

Polypropylene is very similar to high-density polythene but is lighter (it is the lightest known plastic), much less opaque and has greater heat resistance (M Pt 170°C). Squeeze bottles snap back sharply to their original shape, an advantage not possessed by normal polythene containers because of their lower rigidity. Its major disadvantage is brittleness at low temperatures. A new material which can be autoclaved, applications in sterile packaging are likely.

(h) POLYAMIDES (e.g. Nylon)

The outstanding characteristic of nylon is toughness, 15 to 20 per cent of the thickness needed in glass produces a stronger container that is very resilient, e.g. it can be greatly distorted before it bursts and will not fracture if its contents freeze. It is not transparent and its permeability to water vapour is relatively high (both of these are major disadvantages for pharmaceutical uses) but it has good resistance to vegetable oils and many solvents and chemicals. It has excellent heat resistance (M Pt 200+°C) and therefore, can be autoclaved repeatedly at 121°C with no other effect than a slight yellowing due to oxidation, this occurs more readily at 150°C, and dry heat should not be used for its sterilisation.

Unlike the plastics discussed previously, nylon has electronegative polar centres that attract proton donors such as phenols and weak organic acids and bind them to the plastic. Preliminary studies (see Marcus, Kim and Autian (1959) and Kim and Autian (1960) have demonstrated this effect with several common preservatives (e.g. phenol, parahydroxybenzoic acid and its esters, and sorbic and benzoic acids) and have pointed to the danger that concentrations of these substances may be reduced to below effective levels in solutions packed in nylon.

The main applications are for syringes, tubing, clot filters and fittings for giving sets, and packaging films for dressings and instruments.

## 2. Thermosetting Types

(a) PHENOL FORMALDEHYDE

This is used, mixed with fillers to make it less brittle and costly, to produce plastics of the 'Bakelite' type. They are dark and discolour easily, consequently, articles are produced in black or brown. Most types tint food and drink.

Good heat and moisture resistance allow sterilisation by autoclaving but, some types with a mineral filler (e.g. asbestos) are superior to others and should be chosen for the outer caps of injection bottles (see the 'Climbritic bottle') and the caps of eye-drop bottles.

#### (b) UREA-FORMALDEHYDE

This material is also used for closures but is much less heat and moisture resistant than phenol-formaldehyde. Pale or colourless, it can be produced in many colours. It is odourless and tasteless.

#### (c) MELAMINE-FORMALDEHYDE

This has the advantages of both of the previous types and is mentioned here because of its popularity as a bench surface (e.g. Formica) in sterile product units.

From the above information it is not difficult to see why it has not been possible, in the field of sterile packaging, to take full advantage of the strength, lightness and fabrication possibilities of plastics. The disadvantages of lack of thermal resistance, inadequate chemical inertness, permeability to water vapour or oxygen and lack of transparency have, singly or in combination, severely limited the applications of the materials so far produced.

(For general information on plastics, see Child (1955), Melville (1958) and Roff (1956).)

### C TESTING OF PLASTIC MATERIALS

Plastic containers must be carefully investigated before use and, since plasticisers, pigments and other additives may be changed from time to time, it is necessary for manufacturers to check each batch (see Brewer and Bryant, 1960).

For example, because the *United States Pharmacopoeia* recognises the use of plastic bags and assemblies for the collection and transfusion of blood it gives tests for toxicity and pyrogens; these are made on solutions that have been heated with (toxicity test) or passed slowly through (pyrogen test) the assemblies. Similar tests are specified for plastic disposable giving sets (q.v.) in the B.S. Specification for Transfusion Equipment (B.S. 2463 1962).

To these should be added tests for extractives to water (e.g. acid and alkali, chloride, reducing substances and heavy metals) and to the organic solvents used in injections (benzyl benzoate in dimercaprol injection attacks polystyrene syringes (Autian and Dhorda, 1959) and diethyl carbonate, once used in parenteral erythromycin preparations, dissolves the plastic used for the hubs of certain types of dispos-

able needles). Bonding may also need investigation (See also Jammes, 1960).

### D. PROTECTION FROM LIGHT WITH PLASTICS

Carlsen (1954) has discussed the possible use of plastics for protection from light. Because of the ease with which they can be fabricated and coloured it should not be difficult to produce containers of exact thickness, and with the correct extinction curves, in colours that are stable to light and storage. It may be more difficult to ensure that these are inert and, therefore, do not affect the contents. An interesting alternative, suggested by Carlsen, is the use of plastic covers over clear glass bottles, this would overcome the problem of chemical reactivity and, if the design was satisfactory, allow removal of the bottle at intervals for critical inspection for signs of decomposition. Essentials are a transparent plastic coloured with a dye of a type and concentration that will absorb wavelengths of less than 550 m $\mu$  and transmit those above 580 m $\mu$  (see 'Glass'). The potential of plastics in this connexion is shown by the following transmission curve of an amber plastic film marketed by May and Baker Plastics Ltd. as a window blind for protection from light (Fig. 21.8).

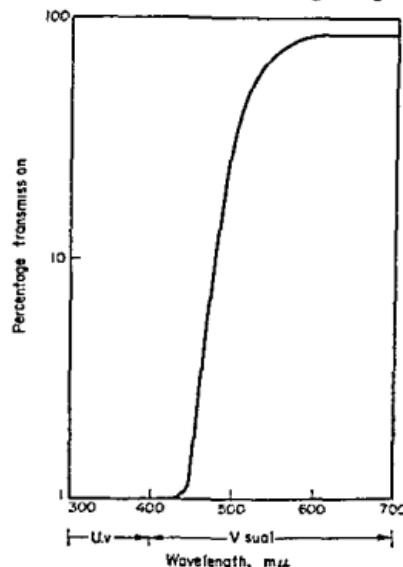


Fig. 21.8 TRANSMISSION CURVE OF A PLASTIC FILM

## CLOSURES

In this section only those parts of the closures of injection containers that are in direct contact with the contents (i.e. wads, plugs and inner caps) will be

considered. Usually these are made from natural or synthetic rubber

### NATURAL RUBBER

Rubber consists of long-chain polymers of isoprene units linked together in the *cis* position. Its most important source is the tree *Hevea brasiliensis* from which a latex, containing 30 to 40 per cent of rubber in colloidal suspension, exudes when shallow cuts are made in the bark.

Some rubber articles are made directly from latex. Before export this is concentrated (e.g. by centrifugation) and ammonia is added (because the rubber particles are negatively charged and, therefore, negative ions stabilise the suspension). However, many articles are made from solid rubber and this is exported in two grades

#### I SMOKED SHEET

The latex is diluted to a standard rubber content and coagulated by adding a little acid (acetic or formic) to neutralise the negative charge. On standing, the rubber separates as a spongy coagulum, which is formed into sheets by passage between rollers, washed to remove coagulant and dried over wood fires. Phenolic substances are absorbed from the smoke and give the material a brown colour, characteristic odour and protection against moulds. This is the type most used in industry; it contains a small amount of non-rubber substances including about 3 per cent resins, 2 to 3 per cent of sugars, salts and proteins.

#### 2 PALE CREPE

The essential differences in preparation are

- (a) The coagulum is much more thoroughly washed, it is torn up and then continually sprayed with water while it is squeezed between rollers. As a result, it has less of the non-rubber constituents
- (b) It is dried at atmospheric temperature and, therefore, is less odorous, but, it lacks the preservative phenols
- (c) Sodium bisulphite is added before coagulation to bleach the crepe to a very pale colour and give protection against moulds.

Pale crepe is used for many medical and pharmaceutical purposes because of its relative freedom from colour and odour.

#### Compounding Rubber

Some of the properties of raw rubber (e.g. its sensitivity to temperature changes and poor elasticity) make it unsuitable for the production of most rubber articles and a large number of substances are added to give better chemical and physical properties and facilitate manufacture, these include—

##### 1 A Vulcanising Agent

The chief disadvantages of raw rubber are—

- (a) Its poor elasticity, if stretched for a long period or outside a narrow temperature range it does not recover completely when released.
- (b) Its strength is poor
- (c) It hardens when cold and becomes soft and sticky when warm.
- (d) It dissolves in many solvents

Vulcanisation increases greatly the range of stress and temperature over which the material is elastic. It also improves its strength and reduces its susceptibility to temperature changes. Vulcanised rubber swells in certain liquids but dissolves in none.

The agent used is sulphur, and during the process this is believed to form cross-links between the long rubber molecules, making it more difficult for them to move and stay apart, therefore, plasticity is decreased and elasticity increased.

There are two methods—

- (a) *Heat Vulcanising* In the absence of accelerators (q.v.) about 8 per cent of sulphur is needed, except for the manufacture of hard rubbers like vulcanite, when 25 to 30 per cent is required. The mixture is heated for about 6 hours at 300°F.
- (b) *Cold Curing* The rubber is treated in the cold with sulphur monochloride, as a vapour or a solution in carbon disulphide. This method can leave traces of hydrochloric acid in the product and is not used for closures and certain medical articles.

## 2 Accelerators

These reduce the time and the amount of sulphur required. Often the product has superior strength and resistance to oxidation. They are organic nitrogenous bases and include thiiazoles (e.g. 2 mercaptobenzothiazole, M B T) thiuarams (e.g. tetramethyl thiuaram disulphide, T M T, which does not need free sulphur) and dithiocarbamates (e.g. zinc dimethyldithiocarbamate, which can vulcanise, with sulphur, at room temperature).

## 3 Activators

These are used to increase the activity of accelerators, e.g. stearic acid or zinc stearate for M B T and zinc oxide for T M T.

## 4 Fillers

Two classes of filler are added to rubber.

*Reinforcing fillers* are used to improve physical properties. For example, carbon black (very finely divided carbon) increases abrasion resistance and tensile strength, the latter can also be increased with finely divided silicates when the colour of carbon black is objectionable. Other materials of this type are zinc oxide and magnesium and calcium carbonates. The most effective substances are believed to combine with the rubber.

*Extending fillers* are added mainly as diluents to reduce cost and, partly, to facilitate manufacture, but sometimes they affect hardness and chemical resistance. Examples are whiting, barytes, talc and asbestos.

## 5 Softeners

These facilitate the incorporation of fillers, make the compound easier and cheaper to manipulate and influence the hardness of the finished product. Pine oil, mineral oils and tar fractions are examples.

## 6 Antioxidants

Rubber is readily attacked by oxygen and as the breakdown is autocatalytic considerable deterioration can occur quite rapidly. The chains are broken at the double bonds and the sulphur cross links, causing softening and weakening until, eventually, all the rubber properties are lost. The oxidation is catalysed by light, heat and traces of copper and manganese. Deterioration can be slowed down by including antioxidants, usually derivatives of aromatic amines and phenols (e.g. phenyl beta-naphthylamine and para hydroxydiphenyl), and, sometimes, agents to sequester copper and manganese.

## 7 Pigments

Originally mineral pigments, such as oxides of iron and sulphides of cadmium and antimony, were used but these are being displaced by coal tar dyes.

## 8 Special Ingredients

- (a) Paraffin wax—this tends to migrate to the surface and produce a protective barrier to oxygen attack and water absorption.
- (b) Rosin—this increases tackiness, e.g. in rubber adhesive for plasters and tapes.

## 9 Lubricants

To assist the removal of closures from their moulds after preparation the rubber compound is dusted with zinc stearate or talc before moulding. A film of these substances usually remains on the articles.

## MANUFACTURE OF RUBBER ARTICLES

The following brief summary of the methods used to make rubber articles may be helpful in further indicating the complexity of rubber manufacture and in explaining why the formulation of a satisfactory rubber for pharmaceutical purposes cannot ignore the problems of processing.

### 1 FROM SOLID RUBBER

#### (a) Mixing

The rubber is softened in a roller mill or a 'dough' mixer. Softeners are added at an early stage and, when the material is sufficiently plastic, accelerators and then pigments and fillers are incorporated. Although the machines are water cooled the sulphur is not added until last because the heat of mixing might start the vulcanisation.

#### (b) Shaping

This depends on the article that is being made.

*Spreading* is used to coat fabrics as in the manufacture of jaconet and battiste. The cloth is passed between a roller and a metal blade on to which the dough, softened with a solvent, is fed. A thin layer of rubber is spread over the surface and after sufficient coats have been applied to give the required thickness the solvent is evaporated by passing the cloth over a heated table.

*Dipping* is used for thin-walled articles like gloves and feeding bottle teats. A former, in the shape of the article, is dipped into the rubber softened with a solvent and the process repeated to give the required thickness.

*Calendering* is the method for rubber sheeting. The rubber is rolled out into a uniform sheet between temperature-controlled steel rollers.

*Extrusion* is one way of producing tubing. The dough is fed into a jacketed tube containing a rotating screw that forces it through an opening of suitable shape. To obtain a satisfactory extrusion rate the rubber base must be rather soft and a large amount of filler is necessary to prevent collapse during vulcanisation. Where filler is objectionable, e.g. for surgical purposes, tubing is formed from sheet.

*Moulding* is used for closures. The dough is placed in the moulds and heat and pressure are used to make it fill the individual cavities.

*Building up*, by hand or mechanically, from parts made by the processes already described, must be used for complicated articles such as air rings. The components are lubricated to prevent adhesion in the wrong places and joined, where necessary, by heat or, after solvent treatment, pressure.

#### (c) Vulcanisation

Heat vulcanisation may be carried out by hot air (for sheeting and coated fabrics) or steam under

pressure (for extruded tubing and built articles). The press heat is often used to vulcanise moulded articles but, occasionally, the complete mould is autoclaved. Vulcanisation in steam gives an article that is more resistant to oxidation.

Cold curing may be used for dipped articles and where heat treatment must be minimised, e.g. some types of tubing and gloves.

#### 2 FROM LATEX

The use of latex eliminates the complicated softening and mixing processes necessary with solid rubber; the inclusions are simply stirred in as solutions or dispersions. The more solid rubber has to be softened, the less well it ages. Latex, which doesn't need this treatment, ages very well. Shaping can be done by spreading or dipping and does not need expensive and inflammable solvents. For some pharmaceutical and medical applications latex has the disadvantages, compared with solid rubber, of absorbing more water and, sometimes, causing skin irritation, both defects are believed due to its relatively high protein content.

(For further information on the technology of rubber, see Haworth, 1953a, b.)

### SYNTHETIC RUBBERS

Attempts to synthesise rubber substitutes have produced several materials that are superior to natural rubber in one or more respects but inferior in others. In general they are—

- (a) More resistant to high and less resistant to low temperatures
- (b) More resistant to the agents that accelerate ageing (light, oxidation and its catalysts, copper and manganese)
- (c) More difficult to process
- (d) More expensive

The method of compounding differs in two major respects. Because synthetic rubbers are harder, more softening is required and esters (e.g. dibutyl phthalate) are used to facilitate this and improve the resilience of the final articles. Because they are more inert, higher concentrations of accelerators and longer vulcanisation times are needed.

The following are four types that have been used or investigated as closure materials for sterile products—

#### 1 BUTYL RUBBERS

These are co-polymers of isobutylene with 1 to 3 per cent of isoprene or butadiene. Polyisobutylene

has no double bonds but the other ingredient provides a few and it is at these that normal sulphur vulcanisation takes place under the influence of accelerators. Carbon black or fine clay are necessary as fillers.

After vulcanisation there are almost no residual double bonds and, consequently, butyl compounds are exceptionally resistant to ageing and chemical attack. Permeability to water vapour and air is very low, water absorption is only slight and their resistance to steam is greater than that of any of the other rubbers, natural or synthetic, mentioned in this section. They are relatively cheap.

Slow decomposition takes place above 130°C, and oil and solvent resistance are not very good.

#### 2 NITRILE RUBBERS (HYCAR)

These are butadiene-acrylonitrile co-polymers. Their most important property is oil resistance, which is due to the polar nitrile group and, therefore, proportional to the amount of acrylonitrile present. They are also heat resistant but this is explained by the unusually efficient protection given by the anti-oxidants present; it does not seem to be a property of the co-polymer itself. Ageing is satisfactory.

Carbon black or fine silica are used to give suitable physical properties

### 3 CHLOROPRENE RUBBERS (NEOPRENE)

These are polymers of 1,4 chloroprene. Because of the chlorine atoms close to the double bonds—

- (a) The bond is less easily attacked by oxygen and, therefore, these rubbers age well. Nevertheless, it is usual to include an antioxidant.
- (b) The polymer is more polar and, consequently, resistant to oils. This is their most important characteristic but nitrile compounds are slightly superior.
- (c) It is not possible to vulcanise (i.e. cross link) the polymer through the double bonds. Therefore sulphur compounds are not used for vulcanisation. This is done with zinc oxide, which attacks elsewhere in the chains. The material is not entirely free from sulphur because stabilisers containing it are sometimes added. Common fillers are carbon black and fine clay.

Heat stability is good and maintained up to 150°C for some grades. Water absorption and permeability are less than for natural rubber. Some compounds

tend to split off hydrochloric acid and it is important to be on guard for this.

### 4 SILICONE RUBBERS

Silicone rubbers were mentioned under the silicone treatment of glass. They are made by polymerisation of methyl silicone fluids, using an inorganic halide as a catalyst, followed by compounding, and then vulcanisation with an organic peroxide. Nitrile groups are sometimes introduced to give improved oil resistance.

Their most important properties are—

- (a) Exceptional heat resistance (up to 250°C), articles have been heated for 100 hours at 180°C without harm.
- (b) Extremely low absorption of, and permeability to, water, as would be expected from their high water repellancy.
- (c) Excellent ageing characteristics, due to their saturated chemical structure (see, for example, Wilkinson *et al.*, 1956).
- (d) Poor tensile strength.

They are very expensive.

(For further information on synthetic rubbers, see Roff, 1956.)

## CHARACTERISTICS OF GOOD PHARMACEUTICAL RUBBER

### 1 Good Ageing Qualities

All types of rubber deteriorate with age and become hard, cracked or sticky as a result of oxidation, but the rubbers used in modern closures are of high quality and under normal conditions of use do not perish for years. In general, latex rubber is better than normal rubber, and synthetics are superior to both. Storage in a cool dark place is desirable since light and heat accelerate deterioration.

### 2 Satisfactory Hardness and Elasticity

The rubber closures of injection vials must be soft enough to allow easy passage of the syringe needle without blunting. In this connexion problems have been created by attempts to improve rubber in other respects. For example, some early types of oil resistant cap contained large amounts of carbon-black filler and were extremely difficult to penetrate with a needle.

As soon as the needle is withdrawn the puncture must close to prevent the entry of micro-organisms and leakage of the contents. Self sealability depends primarily on the elasticity of the closure and is very good for the rubber used for Clinbritic caps (almost 100 per cent pale crepe) which can be penetrated over

100 times without leakage. It is also good for the mix used for the discs and plugs of transfusion bottles, which self seal after perforation with a needle of 2.4 mm diameter (B.S. 2463 1962). However, it is poor for silicone rubber in which the needle holes can often be seen after puncture.

The danger of the needle cutting fragments out of the closure and depositing them in the injection solution, although primarily influenced by the shape of the needle opening (Padgett, 1960), is reduced if the rubber is elastic and not too rigid.

### 3 Resistance to Sterilisation Conditions

Poor quality rubbers become sticky and less resilient after exposure to the conditions of sterilisation but modern closures can be autoclaved many times without significant deterioration. Silicones and some chloroprenes will withstand the dry heat method but it is necessary to select superior types that will not yield toxic substances on repeated sterilisation.

### 4 Impermeable to Moisture and Air

Sealing qualities will depend partly on the adequacy of the overseal holding the rubber to the glass, which will be considered later in this chapter.

Some medicaments, e.g. penicillin salts, are so sensitive to moisture that they are issued dry. In temperate climates most types of rubber produce satisfactory closures but in the tropics several are unsuitable (e.g. natural rubbers) and butyl rubber is preferred because of its low permeability to water vapour.

Injections that are very sensitive to oxygen are best packed in single-dose containers sealed by fusion of glass (ampoules) in which the air has been replaced by an inert gas. If, however, a rubber-closed container, such as a cartridge, is used the rubber should be oxygen impermeable and, again, butyl rubber is the choice. Aqueous suspensions of procaine penicillin illustrate this problem, because if air is not excluded the suspension discolours and becomes viscous (Bloom 1957).

Both these requirements are more easily satisfied if the closure is fairly thick because not only will penetration of water vapour and air be more difficult but self sealing after puncture will be more efficient.

#### 5 Negligible Release of Undesirable Substances

Many of the ingredients used in rubber compounds migrate to the surfaces of finished products producing a bloom. This may be washed from closures by injections, and further extraction may occur as a result of the solution bathing and penetrating the rubber. Consequently, the contents can become coloured, turbid, toxic or partially inactivated.

**Colour** The modern tendency to colour rubber with organic dyes has the disadvantage that sometimes these are more easily extracted than the inorganic pigments used in the past. Colouring matters used in closures must be fast to water at autoclave temperatures.

**Turbidity** Usually the chief cause is mould lubricant (e.g. zinc stearate) but it can also be due to sulphur (left over from vulcanisation), extending fillers (apparently, reinforcing fillers rarely cause trouble probably because they are linked to the rubber matrix), oily material (probably originating from softeners or as an impurity in some grades of carbon black) and reaction between rubber constituents and injection ingredients. Many grades of pharmaceutical and medical rubber offered today have been compounded to minimise this problem. silicone rubbers give little or no trouble. (For further information, see Milosovitch and Mattocks, 1957a.)

**Toxicity** Although there appears to be no evidence that the presence in injections of extractives from rubber closures has caused toxic effects in human beings, the possibility cannot be ignored.

During a long transfusion sufficient of a sclerosing substance has been leached from red rubber tubing to cause thrombophlebitis, a condition that may lead to permanent vein occlusion (Annotation, 1957). The need for care is emphasised also by the fact that the oily substance from some grades of carbon black is carcinogenic. Because of the toxicity of the zinc ion and the wide use of zinc oxide as an activator and zinc stearate as a lubricant, it is essential to see that extracted levels in injections are low (see Reznek, 1953).

The evidence for toxicity of rubber extractives to bacteria (Winner, 1957) and to tissue cultures is more definite. Antioxidants and accelerators have been incriminated and, unless liners and closures are chosen and prepared with great care, experimental work may be spoiled and expensive commercial processes (e.g. virus vaccine production by tissue culture) impaired. Certain silicone rubbers have very low toxicity (Riley and Winner, 1961) but, although liners, bungs and tubing are commercially available, cost severely limits their applications. Special low-toxicity natural rubbers have been compounded for tissue culture work and some of these are being used as closures for injectable preparations.

**Inactivation** Since pH change causes instability in many injections it is important that rubber should not yield acid or alkaline extractives. The *British Pharmacopoeia* specifies heat vulcanisation for closures because cold-curing may leave extractable hydrochloric acid in the rubber. Alkaline extending fillers, such as whiting, could cause serious loss of activity of acid-stable products like insulin.

Catalysis of decomposition by trace metals must not be overlooked, and it is possible that traces of zinc will enhance the oxidative breakdown of certain medicaments. Penicillin solutions have lost activity when transfused through some kinds of rubber tubing and this is believed due to mercaptan compounds produced from the decomposition of accelerators during vulcanisation or (since inactivation was also caused by some accelerator free tubings) to the action of sulphur on the resins found as impurities in most natural rubbers (Bellamy and Watt, 1948). Trial and error have produced suitable closures and tubing for use with penicillin preparations, the plastics polythene and P V C are also quite satisfactory.

#### 6 Negligible Extraction of Injection Ingredients

One of the greatest objections to rubber as a closure material is its ability to remove substances from injection solutions. This is particularly serious when it occurs with preservatives, the concentrations of which may be reduced to ineffective levels.

## BACTERICIDES

There is considerable evidence for the loss of bactericides from solutions packed in rubber closed, multi dose containers and studies have been made of the mechanism of absorption, the influencing factors and possible methods of control. Loss seems to be the result of two processes—

1 *Absorption by the Rubber*

Because rubber has liquid properties it acts as a solvent and, consequently, a preservative in an aqueous injection will be distributed between the two immiscible solvents (water and rubber) according to its partition coefficient. If this strongly favours solution in the rubber serious absorption of the preservative will take place. Table 212, compiled by Royce and Sykes (1957), shows the marked differences between the common bactericides in this respect, some, like phenol and benzyl alcohol, are reasonably satisfactory, while others, e.g. chloro cresol and phenylmercuric nitrate, are very poor

Table 212

Bactericide	Approximate distribution (%) between	
	Rubber	Water
Phenol	25	75
Cresol	33	67
Chlorocresol	85	15
Phenylmercuric nitrate	more than 95	less than 5
Benzyl alcohol	15	85

The ratios in the table are at equilibrium and it is important to appreciate that this is not attained quickly, diffusion throughout rubber is slow, because it is a solid, and full equilibration may take several weeks.

To minimise extraction from injection solutions the B.P. prescribes a method for treating the rubber closures of multiple-dose containers with bactericides before use. After cleaning by washing with a detergent, rinsing with purified water and then boiling in several changes of the latter, the closures are placed in a solution of the appropriate bactericide in a closed container and subjected to saturated steam at 115° to 116°C for a sufficient time to ensure that the whole of the contents are exposed to this temperature for half an hour. Afterwards, the closures are stored in the same solution for at least seven days.

The strength of the bactericidal solution must be at least twice that used in the injection for which the closures are required and the volume must be suffi-

cient to cover the closures and must not be less than 2 ml for each gramme of rubber.

A foolproof method is difficult to devise because the amount of bactericide absorbed by the closure of an injection depends on the type of rubber, the nature of the bactericide, the temperature at which the injection is stored and the presence of substances in solution that increase or decrease the solubility of the bactericide in the rubber. Ideally, the optimum equilibration conditions should be determined separately for each combination of rubber and injection.

2 *Loss by Volatilisation from Outer Surface of Rubber Cap*

If an injection is used within a few weeks, equilibration should prevent significant removal of bactericide but if storage is prolonged the effect of another cause of loss becomes noticeable, the bactericide volatilises into the atmosphere from the outer surface of the closure and is replaced by diffusion from the inner part which then absorbs more from the injection to restore equilibrium. Serious losses can occur in this way and prevention is difficult, but several methods of limitation have been suggested of which two are of particular interest—

(a) *Sealing the Closure with a Less Permeable Material*. Of several techniques tried by Royce and Sykes (1957), including paraffin wax, coated foils and metal overseals, even the best (paraffin wax) only partly prevented the losses. Choice is limited because the protective boundary must not interfere with needle penetration, cause needle blockage (probable with paraffin wax), increase the risk of fragments in the solution or affect the stability of the medicament.

(b) *Reduction of Upper Surface Area and Increase in Thickness of the Closure*. The former will reduce the surface from which volatilisation into the atmosphere can take place, while the latter will increase the depth of the slow-diffusion barrier through which the bactericide must pass to escape.

To limit losses as a whole it would be advantageous to adopt the following procedures—

- (i) Select a rubber with low absorbency for the chosen bactericide.
- (ii) Keep the closure small and of maximum thickness, however, the size of the upper surface must be controlled by the need to keep needle holes well spaced, while increase in thickness must not interfere with needle penetrability.
- (iii) Keep the volume of the injection high in relation to the amount of rubber.

- (iv) Avoid the use of bactericides with unfavourable partitions between rubber and water or that are lost easily by volatilisation and diffusion. The loss of phenylmercuric nitrate is so great (partly due to reaction with the rubber) that its use in multi-dose injections seems undesirable. A search for suitable non-volatile or less volatile preservatives might be profitable.
- (v) Take the loss of bactericide into account when fixing the expiry date of the preparation. For example, the monograph of Heparin Injection B.P. contains a warning that if the injection is kept in a container sealed by a rubber closure a satisfactory concentration of bactericide may not be maintained for more than three years.
- (vi) Reduce the number of doses in the container to ensure rapid use of the contents. The B.P. recommends that a multiple-dose container should not contain an excessive number of doses.

(For further information on this topic, see Lackman *et al.* (1962), Sykes (1958), Wiener (1954) and Wing (1955 and 1956*a, b*))

#### OILS

Because of its liquid properties natural rubber mixes with certain organic liquids, becoming swollen and soft. This occurs to a slight extent with vegetable oils and is very marked with ethyl oleate. Con-

sequently, the B.P. directs that caps for oily injections must be of oil resisting material, e.g. one of the synthetic oil-resistant rubbers.

#### SODIUM METABISULPHITE

Several workers have reported that certain rubber caps reduce the antioxidant activity of sodium metabisulphite by absorbing it or, more probably (Milosovich and Mattocks, 1957*b*), sulphur dioxide from solution. When the caps were soaked in metabisulphite before use the loss of protective activity was much less. This was shown with Adrenaline Injection by West and Whittet (1948). Other work with Morphine Sulphate Injection (Foster, MacDonald and Whittet, 1950) gave conflicting results, early experiments showed a difference in stability with soaked and unsoaked caps but later studies failed to confirm this. A suggested explanation of the apparent contradiction was the change in composition of the rubber of Clinbritic caps that had taken place between the two sets of experiments, originally it was 50 per cent pale crepe, 50 per cent smoked sheet, but at the time of the second experiment it had been altered to 100 per cent pale crepe. The B.P. plays safe by directing that, at least 48 hours before closures are used for injections containing sodium metabisulphite, 0.1 per cent of the reducing agent must be added to the bactericidal solution in which the closures have been equilibrated.

### SUMMARY OF B.P. REQUIREMENTS FOR RUBBER CAPS

- 1 The rubber must be heat vulcanised.
- 2 It must be of good quality, i.e. resist oxidation, allow easy and clean penetration, self-seal and yield no, or only traces, of extractives. In describing injection containers, the *British Pharmacopoeia* states that they must not react with the medicament, yield small solid particles or affect the therapeutic properties of the preparation, all these could be caused by poor quality rubber closures.
- 3 The caps must be cleaned by detergent treatment and then boiled in several changes of purified water before use, to remove mould lubricant, colour and a high proportion of the soluble extractives. It is very difficult to achieve complete freedom from the latter and, even after many boilings and autoclave treatments, the final water may contain oxidisable matter.
- 4 If the caps are to be used for injections containing bactericides or sodium metabisulphite the recommended pre-treatment must be given.
- 5 With regard to the loss of bactericides by diffusion and volatilisation the B.P. simply gives a warning that underlines the difficulty of solving this problem—Rubber closures continually absorb the bactericide from injections in which they are used.\*
- 6 Oil resisting rubber must be used for oily injections.
- 7 The recommendations that multi-dose vials should not contain an excessive number of doses and that the period between the first and last doses should not be unduly prolonged, as well as minimising the chance of bacterial contamination, help to ensure use of the preparation before significant loss of bactericide or antioxidant has occurred.

### TESTS TO CONTROL QUALITY OF RUBBER CAPS

It is not easy to devise satisfactory tests to prove that a particular type of rubber closure is suitable for an

injection product. Many attempts have been made (e.g. Morrissey and Hartop (1957) and Steiger (1956)).

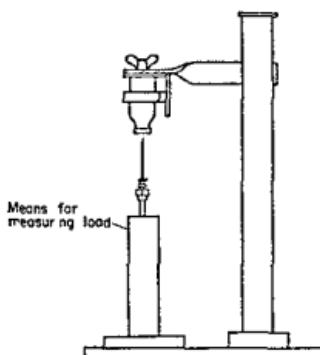


Fig. 219 PIERCING MACHINE  
(from B.S. 3263 1960)

and some particularly important examples are given in the B.S. specifications for rubber closures (B.S. 3263 1960) and transfusion equipment (B.S. 2463 1962). The former includes tests for—

#### 1 QUALITY

The closures must not be tacky after washing in a detergent, rinsing several times, autoclaving for half an hour at 121°C in distilled water and drying for a day at 65°C *in vacuo*.

#### 2 FINISH

They must be substantially free from adventitious dust, fibres, loose particles of rubber, smears of grease and pigment, and quite free from internal foreign matter. Size tolerances are given.

#### 3 PENETRABILITY

The closure is sealed into a vial and the force required to make a hypodermic needle penetrate is measured, using the piercing machine shown in Fig. 219. The *vial is moved up to the needle* at a specified speed. The force must not exceed a stated value.

#### 4 FRAGMENTATION

The piercing machine is used with the vial and needle positions reversed and the vials are half filled with particle free water. Each closure is penetrated five times within a limited area and the last time the needle is washed through to transfer fragments from the bore to the vial. Then the contents are filtered through paper of a colour that contrasts with the rubber and the fragments are counted by eye. The test is carried out on 20 closures, using a fresh needle for each if the previous one has become blunt.

There must not be more than an average of 3 fragments per closure.

#### 5 SELF-SEALABILITY

Two tests are applied. In the first, closed vials, half-filled with water, are inverted and air, equal to the volume inside, is injected. Then the needle is quickly removed and there must be no spray of water from the hole or more than a droplet on the surface. In the second, methylene blue solution is used instead of water and 25 needle punctures are made evenly within a circle of 5 mm diameter. Then the vial is inverted in water in a container to which a prescribed vacuum is applied for half an hour. There must be no signs of leakage in the water or on the closure.

#### 6 WATER EXTRACTIVE

A four hour extract to boiling water is made under reflux and evaporated to dryness. The residue must not exceed the specified amount.

#### 7 ACID OR ALKALI

A specified number of closures are autoclaved with a given volume of freshly boiled and cooled distilled water of pH 6.8 to 7.2. The acid or alkali required for neutralisation of the extract is limited.

#### 8 COMPATIBILITY WITH CONTENTS

Because it is impossible to lay down standards to ensure that particular rubber is suitable as a closure for every injectable product the user must satisfy himself that there is no incompatibility with the preparation in which he is interested. To assist him a method is suggested—

The sterile product is packed aseptically in sterile containers, and several are stored under each of the conditions set out in Table 21.3 half being inverted to give continuous contact between product and closure. An equal number of controls is set up using either vials closed with a satisfactory rubber (preferably) or ampoules.

If the preparation is already known to be unstable at low and/or high temperatures the 4° and 50° tests may be omitted.

At given time intervals up to a year, except for the 50° tests, which are continued for 3 months only, the samples are examined for—

- Foreign insoluble matter, using standard conditions of illumination
- Loss of potency and preservatives, and increase in toxicity (compared with the controls)

Table 21.3

Storage temperature (°C)	Reason
4 (refrigerator)	Chiefly as a check on the original appearance
25	Simulated temperate storage
38	Simulated tropical storage
38 for 16 hr and 4 for 8 hr, alternately, at 90 to 100% R.H.	High humidity can affect penetrability, self sealability and fragmentation
50, or other elevated temperature	Serves as an accelerated test. It may forecast results that will appear later at lower temperatures

(c) Signs of deterioration of the closure, such as sponginess and discolouration. The latter should be checked after drying overnight because absorption of water and certain solutes often produces a bleached appearance. It must also pass the tests of the Standard for penetrability, self-sealability and fragmentation.

If facilities for carrying out compatibility tests are not available, the Standard recommends the use of a closure of natural rubber free from colour and with the minimum of additives, and suggests that a closure manufacturer should be asked for advice (See also Milosovich and Mattocks, 1956.)

#### 9 PERMEABILITY TO WATER VAPOUR

The increase in weight of vials containing dry fused calcium chloride is found after storage under the high humidity conditions of the previous test and compared with the results for containers sealed with

closures known to be satisfactory. Weighings are made fortnightly for 3 months.

Before all these tests, the closures are prepared by the treatments described under the test for quality. To obtain reliable results many replicates are recommended in most cases. Only the bare outline has been given of each test and the Standard must be consulted for full information.

In the Standard for transfusion equipment the discs and alternative seals of transfusion bottles are described. Since these are used for large volume injections (including blood and serum) administered to seriously ill patients, it is particularly important that harmful rubber extractives should not be liberated to the contents. The Standard specifies that the rubber must not yield any substances in sufficient amounts to injure human tissues or inhibit bacterial growth and the colour must be fast to autoclaving. A test for substances inhibitory to bacteria is given and involves the preparation of a blood plate, which is then flooded with a heavy inoculum of a young culture of *Streptococcus pyogenes*. The previously sterilised rubber samples are placed on the surface and incubated at 37°C for 18 to 24 hours. The red blood cells are haemolysed where the organisms are growing and, therefore, there will be rings of unhaemolysed blood around the samples if inhibitory substances are liberated.

#### Other requirements—

- After ageing at 70°C for 168 hr, the closures must self-seal when pierced with the large needles (2-4 mm in diameter) used in transfusion sets.
- They must be of a specified hardness.
- After ageing, as above, and fitting to a transfusion bottle, they must withstand specified exposures to cold and steam under pressure without impairment of function, e.g. they should not become sticky or lose resilience.

## TYPES OF INJECTION CONTAINER

The B.P. contains four requirements for injection containers—

- They must not react with the medicament.
- They must not yield small solid particles.
- They must not affect the therapeutic properties of the preparation.
- They must be sealed to prevent the entry of micro-organisms.

The first three have been discussed already and it is with the last that this section is particularly concerned because the primary aim behind the design of

injection containers is to ensure that their contents are sterile when administered to the patient.

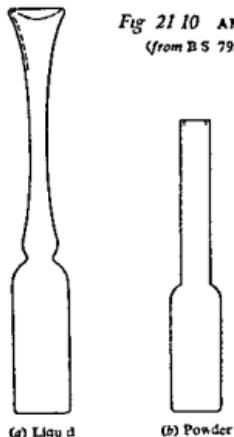
Consideration of containers is simplified by dividing injections into three groups—

- Single-dose injections of small volume
- Single-dose injections of large volume
- Multiple-dose injections

#### A. Single-dose Injections of Small Volume

Each dose is in a separate container from which it is given to the patient with a syringe. The usual volumes are from 0.5 to 10 ml.

**Fig. 2110 AMPOULES**  
(from B.S. 795 1961)



They may be packed in ampoules cartridges or injection units

### 1 AMPOULES

These are the most common single dose containers. They are made entirely of glass in a range of sizes from 0.5 to 50 ml. The glass may be neutral or soda (lime-soda) but the former is usual for parenteral solutions and is essential for those that require containers complying with the pharmacopoeial alkalinity test. A variety of shapes is available (B.S. 795 1961) and Fig. 2110 shows the most important. The powder ampoule is for sterile solids that are unstable in solution and, since the injection is prepared immediately before use, neutral glass is not essential. Figure 2111 illustrates another type of powder ampoule (or tube) of more convenient design for filling with powder, adding the solvent and removing the injection, when the quantity of powder or solution is large.

Because after filling ampoules are sealed by fusion of glass there is no danger of entry of micro-organisms and all the problems associated with the use of rubber are avoided.

Good ampoule glass should melt and seal easily and not splinter excessively when the container is opened which is done by filing at the constriction above the shoulder and, while holding the ampoule as near horizontal as possible, breaking off the neck by bending it away from the file mark. Excessive splintering might contaminate the solution with glass spicules. A popular type of ampoule has a small clean cut (indicated by a coloured spot immediately

above) in the constriction, filing is unnecessary and splintering less.

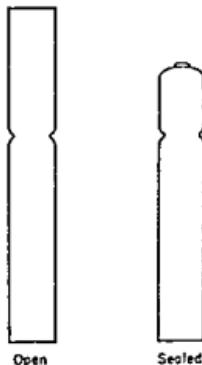
Amber or green ampoules are obtainable for protection against light although it is now more usual to rely solely on packaging in a light tight box.

Ampoules can be used only once and, consequently, they need not be very strong, their walls can be thin with the advantages of lightness, greater resistance to thermal shock and more rapid heat conduction to the contents during sterilisation.

### 2 CARTRIDGES

These are cylindrical glass tubes with a capacity of slightly more than 1 ml. They are closed at one end with a rubber stopper or diaphragm (the latter held in position with a metal overseal) and at the other by a rubber plunger. They are used in a special all metal syringe of which the needle mount is detachable and has a short piercer needle pointing into the barrel. The mount is attached, the syringe plunger is drawn back against a spring and the cartridge is partially introduced into the hollow barrel and pushed against the piercer needle to penetrate the rubber diaphragm. Then the cartridge is fully inserted and the plunger released to hold it firmly. A thread on the syringe plunger screws into a corresponding thread in the rubber plunger of the cartridge, which allows steady pressure on the injection and, if required, slight withdrawal of the plunger to discover if the needle is in a blood vessel (see Fig. 2112). Compared with ampoules—

- (a) They are quicker and easier to use, ampoules have to be opened and their contents carefully measured into a syringe



**Fig. 2111 CONSTRICTED TUBE AMPOULE**

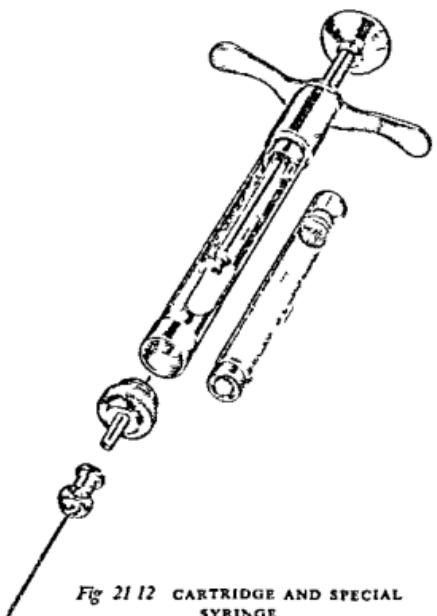


Fig 21 12 CARTRIDGE AND SPECIAL  
SYRINGE  
(Vialite type Courtesy Boots Pure Drug Co Ltd.)

- (b) They are safer, the risk of contaminating the injection during administration is much less and there is no possibility of injecting glass spicules. Some medical personnel develop allergic dermatitis from frequent handling of antibiotic solutions, especially penicillin and streptomycin, accidental contact with these injections is minimised by the use of cartridges
- (c) They are more suitable for suspensions

The syringe is unbreakable, unlike the normal types of syringe used for more conventional containers, and only the needle mount need be sterilised because this is the only part touched by the injection.

Disadvantages are the use of rubber and the need for a special syringe.

### 3 INJECTION UNITS

For many years there has been interest, particularly in the armed services, in disposable injection units combining container and syringe that eliminate the need to transport sterile syringes and/or facilities for sterilisation and, if necessary, permit the safe administration of sterile solutions by personnel with little knowledge of asepsis.

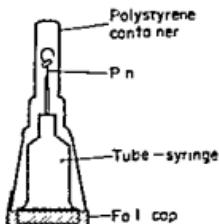


Fig 21 13 TUBE SYRINGE  
(Tubuncle type Courtesy Roche Products Ltd.)

An early development (Fig 21 13) was a soft metal tube with a needle attached. The needle stilette was pressed down to pierce the tube, air was gently expelled, the needle was inserted into the patient and the solution injected by squeezing. This type of unit, which is still in production, has been of particular value for the administration of pain relieving drugs to men in isolated units on active service. It has two major disadvantages, the contents are invisible and it is necessary to guard against the solution corroding the container as well as the container affecting the solution.

Further progress was made possible by the advent of plastics, as the following examples illustrate—

#### *Automatic Injector*

This was developed in America from a method of administering blood used in the Spanish Civil War and it is now marketed by several firms in this country (See Flood, 1958).

The injection is packed in a neutral glass ampoule containing an inert gas under pressure. A length of flexible plastic tubing connects the ampoule to a glass needle mount on the inside of which is a small fabric filter while on the outside is the needle protected by a glass sheath (Fig 21 14).

For use, the sheath is broken from the hub and the needle is inserted into the patient, taking care to keep the injector vertical. Aspiration is performed, to ensure that the needle is not in a vein, by pressing and releasing the plastic tube, blood is seen easily on the filter. Then the neck of the ampoule is broken by bending the plastic tube and the gas expands and forces the liquid into the patient. Glass fragments are retained by the filter.

They have the advantages of cartridges without their disadvantages and, in addition every patient has a brand new needle and the user has nothing to sterilise or assemble, therefore, the time saved and the safety are even greater.

Because gas might be injected into the blood

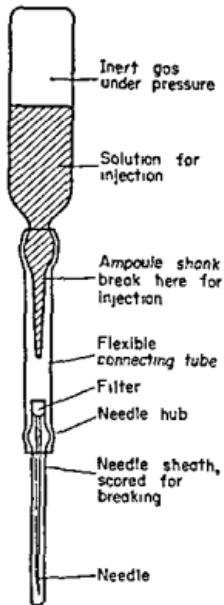


Fig. 21 14 AUTOMATIC-INJECTOR

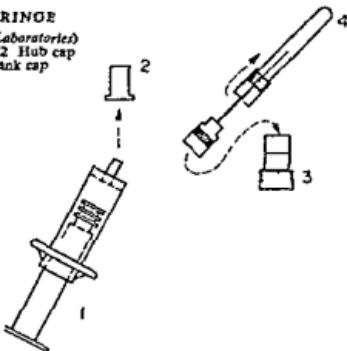
stream, they cannot be used intravenously but it is claimed that if this occurred accidentally there would be no danger from the small amount of gas (about 1 ml) involved.

#### *Plastic Syringes*

In this case the sterile unit consists of the injection in a small plastic syringe. A commercial example, shown in Fig 21 15, has a polythene barrel with the needle protected by a cap of the same material. The plunger is of white opaque polystyrene with a rubber tip. Because of its excellent sealing properties, rubber is superior to plastics at preventing leakage and bacterial contamination. The needle is supplied separately, with hub and shank covered with polythene caps, and has to be aseptically attached to the syringe before use.

The absence of a gas is an advantage, but the contents are not clearly visible and, because the barrel is not rigid, care must be taken not to squeeze it after the cap has been removed, or some of the contents will be expelled prematurely. The use of syringe units made from the plastics at present available is likely to be limited because of the problems listed

Fig. 21 15 PLASTIC SYRINGE  
(Arboject type, Courtesy Abbott Laboratories)  
1 Plastic plunger with rubber tip 2 Hub cap  
3 Needle mount cap 4 Needle shank cap



earlier in this chapter, of which water vapour and/or gas permeability and absorption of preservatives are particularly important.

An ingeniously designed plastic syringe unit has been used in Westminster hospital for the extemporaneous dispensing of an oily injection. The needle is attached to the syringe during manufacture and the plunger is hollow and serves as a needle shield. The sterile injection is filled into the sterilised unit and the barrel sealed with the rubber end of the plunger. For use, the needle shield is removed and its end, which is threaded, is screwed into the rubber, thus making the plunger complete (Fig. 21 16).

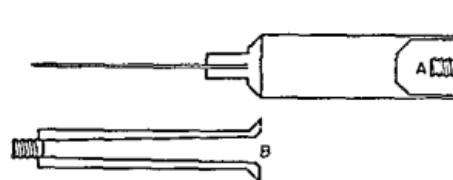


Fig. 21 16 PLASTIC SYRINGE UNIT  
A—rubber stopper and end of plunger  
B—hollow needle shield and shank of plunger

#### 4 ANTIBIOTIC VIALS—see 'Multiple-dose Containers'

##### B Single-dose Injections of Large Volume

Infusion fluids, usually given intravenously, are slowly dripped into the patient's body and 3 or 4 litres may be given in 24 hours. Large containers are necessary and, as these are not cheap, they must be strong enough to withstand frequent cleaning, sterilisation, transport and handling. Although the complete contents may not be used on every occasion it would be dangerous to keep the remainder for a

future infusion because the possibility of contamination during attachment to, or removal from, the transfusion unit is not negligible and bactericides are not permitted in large-volume intravenous injections, therefore, infusion fluids must always be regarded as single dose injections.

#### 1 BRITISH STANDARD TRANSFUSION BOTTLE (B S 2463 1962)

This container, often called a 'blood bottle' because it is used for taking and giving blood, is the most popular for intravenous fluids. Its main features are shown in Fig 21 17.

The graduated capacity is 540 ml, this peculiar volume being the sum of the anticoagulant (120 ml) and blood when the bottle is used for taking blood. Normally only 500 ml of intravenous fluid are included. For strength, it is thick-walled and the sides are inswept at the base. There may be an external groove near to the bottom to take the metal band by which it is suspended over the patient's bed. Moulded on the outside are two scales, one reading from the base when the bottle is upright and the other from the neck when the bottle is inverted. Above the groove is a frosted panel for temporary labelling, e.g. in pencil.

The original blood bottle, many of which are still in use, had a waist that made it easier to hold but more difficult to manufacture and, particularly, to graduate.

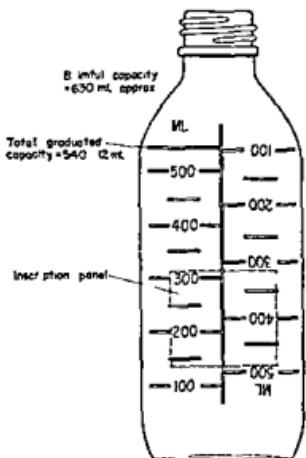


Fig 21 17 TRANSFUSION BOTTLE  
(from B S 2463 1962)

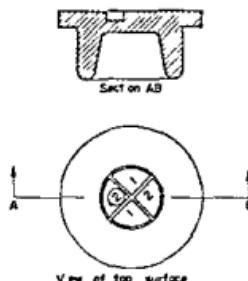


Fig 21 18 RUBBER CLOSURE FOR TRANSFUSION BOTTLE (from B S 2463 1962)

Bottles similar to the B S type, but without the graduations, are sometimes used, and certain hospitals and pharmaceutical firms prefer a litre size. One of the advantages of the latter is the reduction in the number of bottle change-overs and, therefore, of the risk of contamination during a long transfusion.

The B S bottle must not yield, in normal use, any substances with undesirable effects upon the contents (e.g. alkali) or the patient (e.g. flakes). In addition it must comply with tests for

- Thermal Resistance** The bottle must withstand the temperatures of the normal conditions of use. Examples are given in the specification.
- Thermal Shock Resistance** The empty bottle must not break, crack or chip when transferred quickly from a warm water bath to one at least 40°C cooler.
- Resistance to Internal Pressure** When completely filled with water, the bottle must withstand a specified internal pressure.
- Mechanical Strength** The bottle, normally filled, must withstand a prescribed centrifugal force.

The bottle may be closed in two ways—

- With a rubber closure** of the design shown in Fig 21 18 and an aluminium screw cap with a central hole that leaves uncovered the embossed circle on the surface of the rubber.

The circle is divided into four quadrants marked 1 and 2 in diagonally opposite pairs. It indicates the thin region of the closure and prevents penetration of the shank. The numbers 1 and 2 show the piercing areas for taking and giving respectively when the bottles are used for blood. Two needles are necessary for each of these procedures. With the large piercing needles of modern transfusion sets it is unwise to puncture the same quadrant twice because the needle might pass through the previous hole and

not fit well, creating a risk of leakage or contamination during administration. One of the areas marked '2' has a small circular portion thinner than the rest of the piercing areas. This is to facilitate introduction of the closure-piercing needle of the giving set (q.v.). Sometimes the second of the giving areas has a similar thin region for easy penetration by the air-inlet needle. After one use for blood the rubber closures are discarded because all the quadrants have been penetrated. For intravenous fluids, they may be used twice, since only giving is involved, but care is essential to ensure that different pairs of quadrants are used on the two occasions. The British Standard controls the efficiency (i.e. the elasticity) of the rubber closures used for taking blood by requiring that after one puncture in each of two of the quadrants followed by withdrawal of the needle, the closure will maintain a specified pressure and vacuum for 3 days.

(ii) *With a smooth rubber disc and an aluminium screw cap without a hole (Fig. 19 5(4))*

This method is suitable when the preparation is administered by an older type of transfusion unit (now rarely used) and is often preferred when the container is used for the storage of sterile solutions and solvents for such purposes as bathing wounds, bladder irrigation and rinsing the hands during surgery, because the bottle is easier to open. Using the other type of closure, the cap and rubber plug have to be removed separately and it is easy to contaminate the neck while easing out the tightly-fitting plug.

## 2 OTHER CONTAINERS

In the past, intravenous fluids were often packed in conical flasks of resistance glass plugged with long-fibre non absorbent cotton wool. Clip-top milk bottles and large McCartney bottles (very like blood bottles but of thinner glass and narrower neck) were also used. Each has disadvantages, e.g. the closure of the flask, the quality of the glass and rubber of the milk bottle and the narrow neck of the McCartney bottle, but, nowadays, the most serious objection to all three is the need to transfer the contents aseptically to a container that will fit the normal giving set. Only the most exceptional circumstances would justify the bacteriological hazard involved in doing this. However, plugged flasks are useful as bulk containers for liquids requiring aseptic distribution and McCartney bottles are sometimes used for sterile liquids not for parenteral use, and for sterile and non-sterile solutions and solvents required for preparing injections. Milk bottles are obsolete.

The most important alternatives to blood bottles

are the large containers (1 or 2 litres) used by some pharmaceutical houses for their own intravenous fluids. Usually these can be fitted to the normal giving sets but sometimes firms supply their own

### *Giving Sets*

The equipment used for transferring intravenous injections from the bottle to the patient is called a giving, administration or recipient set. There are two main types, one intended for re-use and the other disposable (see B.S. 2463 1962). They both consist of a piece of tubing about 5 ft long with a needle at one end for insertion into the vein and a means of piercing the bottle closure at the other. An inlet to admit filtered air to the bottle as the fluid is withdrawn is provided. Near to the bottle is a drip chamber from which the speed of administration can be estimated, e.g. if the external diameter of the inlet to the chamber is 4 mm a rate of 40 drops/min corresponds to a delivery of approximately 540 ml of blood in 4 hr. The unit must have a filter to prevent the transfusion of clots, from blood, and traces of undissolved material, from plasma and serum. (The latter are usually reconstituted from freeze-dried material immediately before use.)

After use, the non-disposable type is rinsed well and then sent to the Regional Blood Transfusion Centre where the components are separated, thoroughly cleaned, examined for faults, reassembled with freshly sharpened needles and sterilised.

#### *(a) Non-disposable type (Fig. 21 19)*

This is of the general pattern described above and is designed to be used with a bottle sealed with a closure of the type shown at Fig. 21 18. It has a combined drip tube and nylon filter. The bottle is pierced, separately, with the two needles. The one connected to the air filter is long enough to reach above the surface of the liquid when the bottle is inverted.

#### *(b) Disposable types*

A disposable giving set is attractive in several ways—

- (i) The time, tedium and expense involved in cleaning, inspecting and re-assembling the many components of a recoverable set, and in re-sharpening the needles, is saved.
- (ii) Non-disposable sets have to be rinsed well immediately after use, particularly when blood has been transfused because this is very difficult to remove once it has dried. Hospital staff are relieved of this responsibility.
- (iii) The patient is assured of a perfectly clean unit and a new sharp needle.

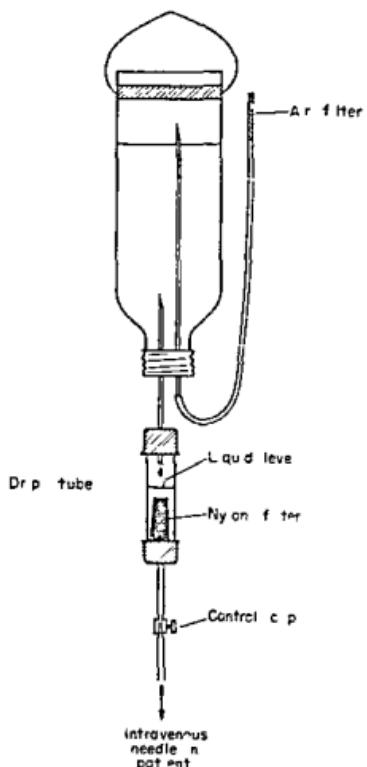


Fig. 21.19 NON DISPOSABLE GIVING SET

- (iv) The incidence of thrombophlebitis after long transfusions is very much less when plastic, instead of red rubber, tubing is used.

For the last reason, particularly, disposable sets have been introduced in certain parts of the country although the overall cost of recoverable types is lower at present.

Figure 21.20 illustrates a commercial disposable recipient set for blood, manufactured by Capon Heaton & Co Ltd, Birmingham, 30. The main features are—

- (i) The chief construction material is semi flexible PVC, and all joints are high frequency welded to avoid the use of cements or solvents that might leave toxic residues. The piercing needle, filter and adaptors are of nylon.

- (ii) Moulded into the piercing needle is a long metal one that serves as an air inlet and is connected to a cotton wool filter.
- (iii) Below the needles is a double chamber, the upper contains the nylon filter the heat-sealed edge of which is covered with PVC to prevent the detachment of tiny globules of fused nylon during transfusion, the lower is smaller and is used to observe the rate of drip.
- (iv) On the PVC delivery tube is a flow-control consisting of a roller that works along an inclined guide to compress or release the tube.
- (v) Immediately behind the intravenous needle is a short length of rubber tubing. This is necessary because, sometimes, drugs are administered during a transfusion by injection through the wall of the tube. PVC does not seal effectively afterwards.
- (vi) The intravenous needle is separately packed in a test tube and is attached, by a piece of

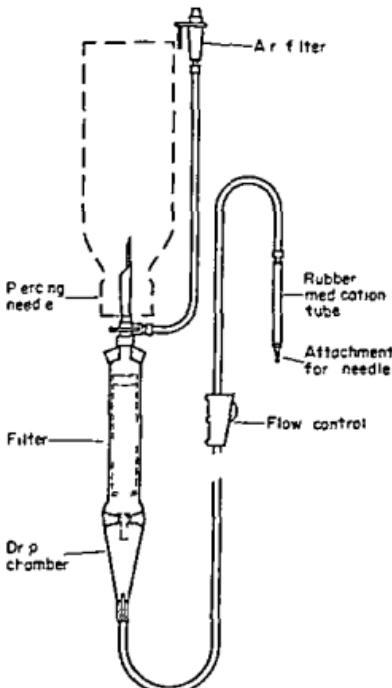


Fig. 21.20 DISPOSABLE GIVING SET  
(Courtesy Capon Heaton & Co Ltd.)

- plastic tubing to a female adaptor that is connected before use to its counterpart at the end of the delivery tube
- (vii) The piercing needle and all other couplings are protected by nylon caps that cannot be replaced after removal. If any of these caps are loose or missing it is unsafe to use the unit
- (viii) The units are sterilised at 20 p s i g for 20 min, and samples are regularly tested for sterility, toxicity and pyrogenicity

A set for intravenous fluids is also available. It differs in two respects only, there is no filter chamber but between the piercing needle and the tubing is a small safety filter for removing particles, e.g. rubber fragments, produced by insertion of the needles, and clots, if the set is used in error for blood or blood derivatives.

An outline of the use of the disposable blood set will indicate the relative complexity of this method of administering an injection—

- The contents of the bottle are mixed by several inversions
- The sheath is removed from the piercing needle which is then forced through the closure without twisting
- The flow control is closed, the bottle suspended over the bed and the air filter hooked in a position higher than the fluid level, the cap of the filter is then removed
- The upper chamber is compressed and released until filled with fluid and this is repeated for the drip chamber until it is about  $\frac{1}{2}$  full. Only the latter applies to the intravenous fluid set
- The flow control is opened and the fluid allowed to expel air from the tube
- The intravenous needle is aseptically unpacked, inserted into the vein and then joined to the delivery tube
- The flow is adjusted to the required rate

(For further information see Farquhar, Shannon and Batchelor (1961) and Jenkins *et al* (1959))

#### Plastic Bags

The U.S. Pharmacopoeia mentions plastics as alternative containers for blood and plasma because for some years polyvinyl chloride bags have been used in America for these fluids. Their advantages over glass bottles include lightness, slightly smaller storage volume, disposability and, according to some authorities, prolongation of the life-span of red blood cells. The difficulty of estimating the volume in a non-rigid, slightly elastic container and, particularly, the cost, have limited their development in

this country and restricted their use to a few special applications, e.g. the collection of high platelet blood (because platelets adhere to glass). One British manufacturer markets an infusion fluid in a plastic bag connected to its own plastic giving set (See Dudley *et al* (1958) and Simpson (1961)).

#### C. Multiple-dose Injections

With a multiple-dose container it is more difficult to comply with the official requirement that an injection must be dispensed in a container sealed to exclude micro-organisms. As a result, there have been several important changes in design over the years and it is instructive to consider these in detail.

##### 1 RUBBER CAPPED VIAL

It is not permissible to withdraw from an injection container successive doses on different occasions simply by removing the cap. The risk of contaminating the solution would be far too great. The aim must be to prevent altogether the entry of unsterile air, which is facilitated if the closure can be penetrated by a syringe needle and, therefore, left in position. A further essential, that the material must re-seal when the needle is removed, virtually limits the choice to rubber.

The earliest multi dose vials consisted of glass bottles ranging from 5 to 30 ml with a fairly wide mouth covered by a rubber cap that was wired tightly in position (Fig. 21.21). These had two major disadvantages—

(a) The cap was constantly exposed to dust (and, therefore, to micro-organisms) and to the deleterious action of light and air.

(b) It was difficult to sterilise the finally packed injection because when the bottle was heated its contents expanded and the cap ballooned, burst or (if it had not been wired) blew off. During ballooning, loss of elasticity occurred from the stretching of

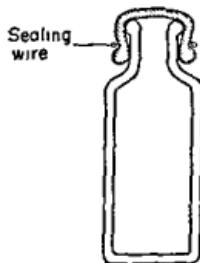


Fig. 21.21 RUBBER CAPPED VIAL

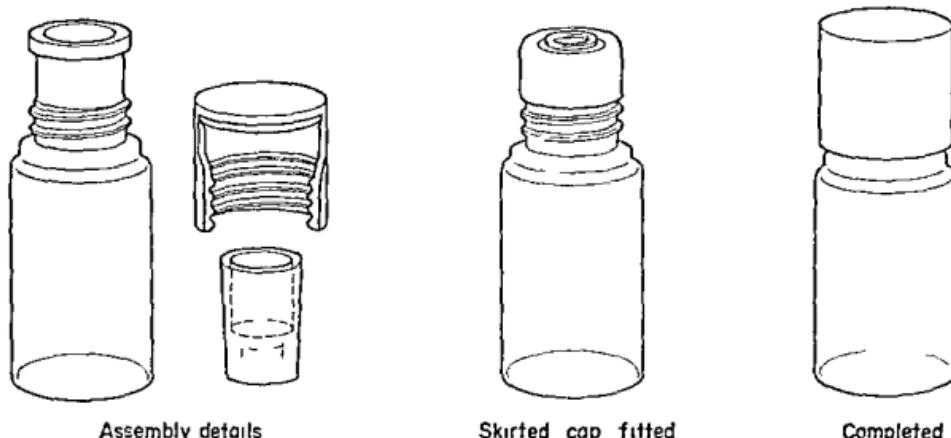


Fig. 21.22 CLINBRITIC BOTTLE, MARK 1

Plastic ring not shown  
(Courtesy Britton Malcolm & Co. Ltd.)

the rubber under heat and, on cooling, the surface became concave, making even spacing of the needle insertions difficult and encouraging the use of the bottom of the concavity, with the danger of producing large enough holes to admit bacteria. Several methods were devised to overcome this difficulty but, since they involved the use of vents, clamps or aseptic technique, they were not very acceptable as long-term solutions.

## 2 CLINBRITIC BOTTLE (Mark I)

In 1937 a considerable advance was made by the production of a new style container (Fig. 21.22) in accordance with the suggestions of Berry (1937, 1938). It comprises—

(a) A bottle of neutral glass, either clear or amber, and in four sizes (10, 25, 50 and 100 ml approx.)

(b) An inner rubber cap consisting of a thick-walled plug with a thin central diaphragm, and a comparatively thin-walled 'skirt' above. The cap is pushed into the bottle until the diaphragm is flush with the neck and the skirt is then brought down over the outside which it grips tightly.

(c) An outer bakelite screw cap, with three functions—

(i) To hold the rubber cap securely and prevent its expulsion or deformation during sterilisation. This is assisted by a thick liner backed with rubber and faced with an absorbent pad.

(ii) To protect the surface of the cap from dust and droplet infection from the atmosphere. In addition to the physical protection given by the cap a degree of chemical protection is possible because the absorbent facing of the liner can be moistened with an antiseptic solution (Solution of chloroxylenol B.P. 10 per cent, Glycerin 10 per cent, Water for Injection 80 per cent, has been suggested) to which organisms left on the cap after use will be constantly exposed. Present-day knowledge of the ability of rubber to absorb preservatives prompts some speculations on this procedure. Absorption of the antiseptic, followed by diffusion to the inner surface of the cap could lead to traces in the injection, and although the effects (e.g. on toxicity and stability) might be negligible it is difficult to countenance this extra, potential rubber extractive. A more acceptable method would be to moisten the liner with a solution of the bactericide used in the injection, when no inward diffusion would be expected with equilibrated caps. In fact, it seems doubtful if bacteria could multiply on equilibrated caps, in which case, the value of any antiseptic solution is questionable except, possibly, when non-treated caps are permissible, e.g. for injections (such as Leptazol) for which no bactericide is required. A further point arises from the use of the faced rubber liner in close contact with the rubber cap, bactericide might diffuse from the cap into the liner (especially if the facing was moist) and increase the loss from the solution—in which case it would be desirable to equilibrate the liner too.

(iii) To protect the rubber cap from the deleterious effects of light

The closure of this bottle has a serious weakness, it is possible to remove and replace the rubber cap, and even the contents, without the removal being detectable. In the past, nurses and doctors sometimes transferred an injection to a flamed gallipot (a tiny basin) to facilitate filling the syringe, and a safeguard is essential to discourage and immediately detect this hazardous malpractice. A simple method is to seal the rubber cap and bottle junction with a 'Visking'. This is a plastic ring that is flexible when moist and, in this condition, easily slipped over the neck. On drying it contracts and hardens to grip the cap and bottle thread tightly. It is not thick enough to interfere with the fitting of the bakelite cap. Normally, anyone wishing to remove the rubber cap would tear off the ring first when its absence would warn others that the contents were no longer safe to use. Provided nursing and medical staff are made aware of the significance of the seal, the method is very successful. If an antiseptic solution is added to the cap only a drop or two should be used to avoid softening and loosening the ring.

### 3 ANTIBIOTIC VIAL

When penicillin was introduced to medicine pharmaceutical firms were faced with the problem of packing a substance that was—

(a) Very unstable in solution and therefore, had to be supplied as a sterile solid to which sterile solvent was added shortly before use

(b) Given to individual patients up to six times a day, often for several days. This weighted the choice of container in favour of a multi-dose vial, particularly as the antibiotic was stable in solution for several days in a refrigerator

(c) In very great demand. Consequently, the manufacture of a special vial (Fig. 21.23) was practicable

It was especially important to prevent bacterial contamination of the contents, not only to protect the patient from harmful penicillin resistant species but, also, to avoid destruction of the penicillin by penicillinase-producers. Therefore, particular attention was paid to the seal.

The main features were—

(i) A small glass vial of only a few millilitres capacity and with a flanged neck

(ii) A small rubber plug with a short, hollow shank and a larger flat top. The shank was pushed into the mouth of the vial until the top was pressed firmly on the neck

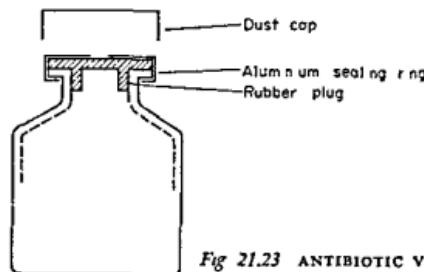


Fig. 21.23 ANTIBIOTIC VIAL

(iii) An aluminium sealing ring shaped tightly to the bottle flange and the top of the plug but leaving a hole at the centre of the latter for penetration of the needle. This type of seal has four advantages, (1) it is not easily damaged, (2) obviously it is not meant to be removed, (3) removal is difficult, and (4) removal cannot be achieved without tearing the ring which, therefore cannot be replaced. The rubber at the central hole was sometimes protected by covering the complete closure with an aluminium dust cover that was easy to remove and replace.

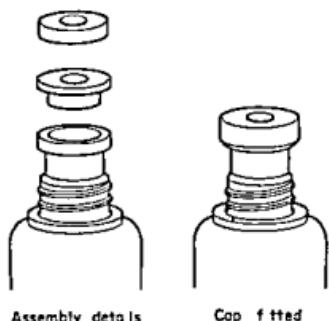
### 4 CLINBRITIC BOTTLE (Mark II)

The seal of the antibiotic vial is much more reliable than that of the Clinbritic bottle (Mark I). A determined person can often remove the skirted cap and plastic ring without damaging the latter and even, though this is more difficult, replace them with the ring intact. It is inconceivable that anyone would deliberately open and reseal a bottle in this way but the fact that it can be done is disturbing.

Appreciating the advantages of a more secure closure, the manufacturer of the Clinbritic bottle (Britton Malcolm & Co Ltd, S.E.1) has produced a new pattern (Fig. 21.24) in which the skirted cap is replaced by a plug of the antibiotic vial type and the flange of the bottle is deeper, to provide a grip for an aluminium sealing ring. Manufacturers, using costly machinery, spin the rings on to antibiotic vials, for the user of small numbers of Clinbritic bottles there is a closing tool that turns the lower edge of the ring under the flange.

### RECENT DEVELOPMENTS IN THE ANTIBIOTIC VIAL

The antibiotic vial, in sizes that usually range from 2 to 25 ml, is used extensively for antibiotics, therapeutic substances and other injections, and for solids, solutions or suspensions. It has displaced completely the simple rubber-capped vial which



*Fig. 21.24 CLINBRITIC BOTTLE, MARK 11*

Bakelite cap omitted  
(Courtesy Britton, Malcolm & Co. Ltd.)

continued in use long after the invention of the Clinbritic, because of the higher cost of the latter

On the whole, antibiotic vials and Clinbritic bottles have complementary applications. The former are preferred by manufacturing houses because they are relatively inexpensive (desirable in a container that is discarded after use) and usually light in weight (valuable when transport over long distances, including abroad, is necessary). The latter are popular for the extemporaneous dispensing of injections in hospitals, because the bottles are strong and stand up well to frequent re-use.

Sometimes the smaller vials of antibiotic powders contain only one dose, i.e. are used as single-dose containers. One of the reasons for this is that vials are easier than ampoules to machine-fill with powder. The advantages over multi-dose packs include absence of the contamination risk, from the withdrawal of successive doses, and limitation of the contact between solution and rubber to a few minutes before administration.

Much effort has been devoted to improving antibiotic vials—

(a) Neutral, boro-silicate and sulphured glass (the latter for sterile powders) have been used and silicone treatment is common for suspensions.

(b) Among synthetic rubbers, butyl is particularly valuable because of its low moisture and gas permeability. Plastics have been disappointing, because they are less elastic than rubber, it is more difficult to get a good fit between the plug and the bottle, and some types are highly permeable to phenolic bactericides and sodium metabisulphite.

(c) Recently, a new type of rubber closure has been introduced. In the old pattern the hollow of the shank is difficult to clean and the thin area for needle puncture is too small and can lead to penetration of the walls of the shank, thus increasing the risk of fragmentation. Neither of these disadvantages applies to the new closure, which is a flat disc of rubber that rests on the neck and is secured by the conventional overseal.

#### Single Dose v. Multi-dose

On the whole, multiple dose containers are more popular than ampoules with the medical and nursing professions. Possible reasons are—

- (a) They are more convenient when the dose has to be varied.
- (b) Ampoules are more difficult to manipulate (snap-open types have not been available for long) and require a greater degree of aseptic technique.

However, consideration of the following disadvantages of multi-dose containers leaves no doubt about the superiority of single-dose packs, particularly ampoules—

(a) The contents may become contaminated each time the container is used and, although a bactericide must be present (itself a disadvantage) it would be rash to assume that it will control every possible contaminant. The danger is increased by the sharp in-drawing of air that occurs, to replace the withdrawn dose, as the needle is removed, and the prac-

tice of some users of injecting a syringeful of air to facilitate removal of the solution.

(b) Their closures are less efficient than fusion of glass at excluding micro-organisms, air and moisture and at retaining the gases (e.g. nitrogen) used for stabilisation. Also they are more likely to allow loss of solvent with consequent concentration of the solution.

(c) Rubber is essential, because of its exceptional qualities of easy penetrability and efficient re-seal, but it provides many problems such as extractives and absorption. However, some of these are less important when the content is a powder.

(d) Wrong measurement of the dose is more likely than with a single-dose container.

(e) They can be more wasteful, it is sometimes necessary, e.g. for reasons of stability, to discard containers before all the solution has been used.

It is understandable, therefore, that official sources should discourage the use of multi-dose containers.

unless the number of doses is small and the contents are used quickly. This view is reflected by the increasing number of injections for which the *British Pharmacopoeia* directs the use of ampoules.

## REFERENCES

- 1 ANNOTATION (1957) Tubing for intravenous infusions *Lancet* 1, 627
- 2 AUTIAN, J. and DHORDA, C. N. (1959) Evaluation of disposable plastic syringes as to physical incompatibilities with parenteral products *Am J Hosp Pharm* 16, 176-179
- 3 BELLAMY, L. J. and WATT, C. H. (1948) Factors involved in the deactivation of penicillin solutions by rubber tubing *Nature, Lond* 161, 940-942
- 4 BERRY, H. (1937) Rubber caps for vaccine bottles *Pharm J* 138, 397-398
- 5 BERRY, H. (1938) Rubber capped vaccine bottles *ibid* 140, 627
- 6 BERRY, H. (1953) Pharmaceutical aspects of glass and rubber *J Pharm Pharmacol* 5, 1008-1017
- 7 BLOOM, C. (1957) Packaging of pharmaceuticals *Pharm J* 178, 207-209
- 8 BRADLEY, D. C. (1953) Chemistry, properties and uses of silicones *Chem & Drugg* 159, 41-43
- 9 BREWER, J. H. and BRYANT, H. H. (1960) The toxicity and safety testing of disposable medical and pharmaceutical materials *J Amer pharm Ass, Sci Ed* 49, 652-656
- 10 BRITISH STANDARD 795 1961 *Ampoules* British Standards Institution, London
- 11 BRITISH STANDARD 2463 1962 *Transfusion equipment for medical use* British Standards Institution, London
- 12 BRITISH STANDARD 3263 1960 *Rubber closures for injectable products* British Standards Institution, London
- 13 CARLSEN, T. (1954) Requirements for a container giving protection against light *Dansk Tidsskr Farv* 28, 84-89 (Abstracted in M & B Pharmaceutical Bulletin (1955) 4, 47-48)
- 14 CARLSEN, T. (1957) The spectral distribution of the light sensitivity of some chemicals and the use of light protective containers *ibid* 31, 182-201 (Abstracted in M & B Pharmaceutical Bulletin (1958) 7, 47-48)
- 15 CHILD, C. L. (1955) Symposium on plastics in pharmacy *Nature and properties of plastics J Pharm Pharmacol* 7, 793-805
- 16 DIMBLEBY, V. (1953) Glass for pharmaceutical purposes *J Pharm Pharmacol* 5, 969 989 and 1022
- 17 DUDLEY, H. F., RICHMOND, J., MCNAIR, T. J., PATON, B. C. and CUMMING, R. A. (1958) Plastic bags for storing and transfusing blood *Lancet* I, 294-296
- 18 PARQUHAR, J. W., SHANON, D. W. and BATCHELOR, A. D. R. (1961) Equipment for intravenous fluid therapy of children *Lancet* 2, 537-538
- 19 FERGUSON, W. C. (1948) Polytetrafluoroethylene *Chem & Ind Aug 20th* 586-590
- 20 FLOOD, C. M. (1958) An injection unit instead of the hypodermic syringe *Lancet* 2, 1114-1116
- 21 FOSTER, G. E., MACDONALD, J. and WHITTET, T. D. (1950) The stability of injection of morphine sulphate *J Pharm Pharmacol* 2, 673-683
- 22 GIRARD, P. and KEMY, G. (1950) *Ann Pharm Franc* 8, 462
- 23 GREENE, C. H. (1961) Glass *Sci Amer* 204(1), 92-105
- 24 HARRIS, D. K. (1962) Polytetrafluoroethylene *Lancet* 1, 587
- 25 HAWORTH, J. (1953a) Symposium on containers and closures The technology of rubber *J Pharm Pharmacol* 5, 990-1007
- 26 HAWORTH, J. (1953b) Rubber manufacture *Alchemist, Leeds* 17, 274-280 and 361-366
- 27 HEATHCOTE, J. and WILLS, B. A. (1962) Hydrolytic destruction of thiamine especially in the presence of cyanocobalamin *J Pharm Pharmacol* 14, 232-236
- 28 JAMINET, FR. (1960) The use of plastics in the packaging of pharmaceutical solutions Part II *Il Farmaco (Ediz Pract)* 15, 639-668 (Abstracted in M & B Pharmaceutical Bulletin (1960) 10, 60)
- 29 JENKINS, W. J., STONE, B., KNOWLES, G. S. A., TOVEY, G. H. and SHARPE, R. A. (1959) Experiences with a disposable plastic transfusion and giving set *Lancet* 1, 139
- 30 KIM, H. K. and AUTIAN, J. (1960) Binding of drugs by plastics 2 Interaction of weak organic acids with plastic syringes *J Amer pharm Ass, Sci Ed* 49, 227-230
- 31 KONDRTZEP, A. A. and ZVIRBLIS, P. (1957) Stability of atropine in aqueous solutions *ibid* 46, 531-535
- 32 LACKMAN, L., WEINSTEIN, S., HOPKINS, G., SLACK, S., EISMAN, P. and COOPER, J. (1962) Stability of antibacterial preservatives in parenteral solutions I Factors influencing the loss of antimicrobial agents from solutions in rubber stoppered containers *J Pharm Sci* 51, 224-232
- 33 LEVIN, R. (1958) *The pharmacy of silicones* Chemist and Druggist, W.C.2
- 34 LOEWENSTEIN, K. L. (1948) Glass *Science News* 8, 85

- 35 MARCUS, E., KIM, H. K., AUTIAN, J (1959) Binding of drugs by plastics I Interaction of bacteriostatic agents with plastic syringes *J Amer pharm Ass, Sci. Ed.*, 48, 457-462
- 36 MELVILLE, H. (1958) *Big molecules* G Bell, London
- 37 MILOSOVICH, G., and MATTOCKS, A. M. (1956) Sorption of water by rubber closures for injections I Effect of inorganic salts *J Amer pharm Ass, Sci Ed* 45, 758-764
- 38 MILOSOVICH, G., and MATTOCKS, A. M. (1957a) Haze formation of rubber closures for injections *ibid.* 46, 377-381
- 39 MILOSOVICH, G. and MATTOCKS, A. M. (1957b) Sorption of water by rubber closures II Effects of vapour pressure, bisulphite and washing treatments *ibid.* 350-354
- 40 MORRISEY, E. J. and HARTOP, W. L. (1957) Extraction tests for rubber closures *Drug Standards* 25, 1-4
- 41 MOXEY, P. (1959) An introduction to high density polythene *Rubber and Plastics Age* 40, 505
- 42 NIELSEN, A. T. (1959) The stability of posterior pituitary injections during storage *Dansk Tidsskr Farm* 33, 1-9
- 43 PADGETT, W. (1960) The fragmentation of rubber closures by hypodermic needles *Pharm J* 184, 471-472
- 44 REZNICK, S. (1953) Rubber closures for containers of parenteral solutions I The effect of temperature and pH on the rate of leaching of zinc salts from closures in contact with (acid) solutions *J Amer pharm Ass, Sci Ed* 42, 288-291
- 45 RILEY, I. H. and WINNER, H. I. (1961) A note on bacteriological toxicity tests of silicone rubbers for medical and pharmaceutical uses *J Pharm Pharmacol* 13, 111-114
- 46 ROFF, W. J. (1956) *Fibres, Plastics and Rubbers* Butterworth, London
- 47 ROYCE, A. and SYKES, G. (1957) Losses of bacteriostat from injections in rubber closed containers. *J Pharm Pharmacol* 9, 814-822
- 48 SIMPSON, D. C. (1961) Warning device for intravenous therapy *Lancet* 2, 530
- 49 STEIGER, K. (1956) Containers and closures for injection solutions *Publ Pharm* 13, 75-79
- 50 STEVELS, J. M. (1960/61) New light on the structure of glass *Philips tech Rev* 22, 300-311
- 51 SWARTZ, C. J., LACHMAN, L., URBANYI, T., and COOPER, J. (1961) Colour stability of tablet formulations IV. Protective influence of various coloured glasses on the fading of tablets *J Pharm Sci* 50, 145-148
- 52 SYKES, G. (1958) The basis for "sufficient of a suitable bacteriostatic" in injections *J Pharm Pharmacol* 10, 40-45T
- 53 THOMAS, D. M. E. (1953) Chemistry, properties and uses of silicones *Chem & Drugg* 159, 114
- 54 THOMAS, D. M. E. (1954) Silicones and their use in medicine and pharmacy *Alchemist, Leeds* 18, 21-24
- 55 WEST, G. B. and WHITTET, T. D. (1948) The stability of adrenaline solutions Part V Further observations on the storage of solutions containing hydrochloric acid, tartaric acid and ascorbic acid. *Quart J Pharm* 21, 225-228
- 56 WIENER, S. (1954) The interference of rubber with the bacteriostatic action of thiomersalate *J Pharm Pharmacol* 6, 118-125
- 57 WILKINSON, J. F., FREEMAN, G. G., NEW, N. and NOAD, R. B. (1956) Silicone rubber tubing in blood transfusion work. *Lancet* 2, 621-624
- 58 WING, W. T. (1955) An examination of rubber used as a closure for containers of injectable solutions Part I Factors affecting the absorption of phenol. *J Pharm Pharmacol* 7, 648-658
- 59 WING, W. T. (1956a) *ibid.* Part II. The absorption of chlorocresol *ibid.* 8, 734-737
- 60 WING, W. T. (1956b) *ibid.* Part III. The effect of the chemical composition of the rubber mix on phenol and chlorocresol absorption. *ibid.* 738-743
- 61 WINNER, H. I. (1957) A note on the antibacterial effect of processed natural rubber *J appl Bact* 20, 88-89
- 62 WOKES, F. (1932) Pituitary extract B P *Pharm J* 129, 475
- 63 YEH, S. Y. and LACH, J. L. (1961) Stability of morphine in aqueous solution. *J Pharm Sci* 50, 35-42



## Sterilisation by Heat

METHODS of sterilisation can be divided into two main groups, physical and chemical. Physical methods include heat (dry or moist), ultra-violet light, ionising radiations and filtration through a bacteria-proof filter. In chemical methods, either liquid or gaseous sterilising agents are used. However, to relate these more conveniently to the rest of the material in this book it is useful to classify them in another way—

*Methods Widely Applied to Pharmaceutical Preparations* These include heat, filtration and a combined physical and chemical method involving heat in the presence of a bactericide. They will be discussed in this and the next five chapters.

*Methods Mainly used for Surgical Materials and Equipment* These include ionising radiations and gaseous sterilisation which are more conveniently discussed separately.

Liquid sterilising agents are not extensively used for either of the above purposes and are more suitably studied with antiseptics and disinfectants (see 'Preface').

### DRY HEAT

All micro-organisms, including bacterial spores, can be destroyed by heat. The mode of action is imperfectly understood but differs for dry and moist heat. With dry heat the chief cause of death is oxidation (Rahn, 1945).

Sterilisation by dry heat is usually carried out in an apparatus known as a hot-air oven in which heat is transferred from its source to the load by radiation, convection and, to a lesser extent, conduction. This method will be described in detail in the following sections. For syringe and instrument sterilisation special sterilisers, in which radiation or conduction are the primary agents of heat transfer, have been developed and these are discussed briefly in chapter

Pharmaceutical interest in ultra-violet light is due mainly to its use for reducing the bacterial count of air, and it will be considered in this connection in the section dealing with asepsis rooms.

#### METHODS FOR PHARMACEUTICAL PREPARATIONS

The *British Pharmacopoeia* has five methods for ensuring that injections are sterile—

- 1 Dry heat
- 2 Moist heat
- 3 Moist heat in the presence of a bactericide
- 4 Filtration through a bacteria-proof filter
- 5 Aseptic technique during preparation

For success, the last two require a much higher degree of skill than the others and, therefore, are not discussed in this chapter.

(Valuable information on sterilisation principles and techniques will be found in Perkins (1956), Reddish (1957) and Sykes (1964).)

29 Flaming, i.e. holding in a naked Bunsen flame, is very important in aseptic technique and its applications are illustrated in chapters 24 to 26.

The first stage in the design of a heat sterilisation process is the choice of suitable temperatures and times. These depend primarily on the need to obtain a sterile product but are influenced also by the stability of the preparation or material and, consequently, it is not always possible to lay down a single set of conditions that are always applicable.

Published information on the exposures necessary to kill pathogens by dry heat shows that while  $1\frac{1}{2}$  hr at  $100^{\circ}\text{C}$  will destroy all vegetative bacteria, 3 hr at  $140^{\circ}\text{C}$  is needed for the most resistant spores. Mould

spores are killed by  $1\frac{1}{2}$  hr at  $115^{\circ}\text{C}$ , and while the susceptibility of most viruses is similar to that of vegetative bacteria, exceptionally they may be as resistant as bacterial spores, e.g. the virus of homologous serum jaundice. It could be concluded that  $140^{\circ}\text{C}$  for 3 hr would be a satisfactory time-temperature relationship.

However, long processes are unpopular in practice because they reduce the number of times that the steriliser can be used each day. Shorter times at temperatures high enough to give equivalent lethal exposures are preferred. For example, bacteriologists generally use  $160^{\circ}\text{C}$  for 1 hr for sterilising their equipment, and the same treatment is recommended in the Medical Research Council Report on the sterilisation of syringes (1962).<sup>\*</sup> Evidence in favour of exposure at this temperature was presented by Darmady, Hughes and Jones (1958). Using *Clostridium tetani* which they considered to be the pathogen most resistant to dry heat, thermal death times (see p. 316) were determined at temperatures from  $150$  to  $190^{\circ}\text{C}$ . The results were 1 min at  $180^{\circ}\text{C}$ , 5 min at  $170^{\circ}\text{C}$ , 12 min at  $160^{\circ}\text{C}$  and 30 min at  $150^{\circ}\text{C}$ . They suggested the addition of a safety factor of 50 per cent and, to allow for the temperature variations in hot air ovens, the use of the time for the temperature  $10^{\circ}\text{C}$  lower. For  $160^{\circ}\text{C}$  this gives an exposure of 45 min and therefore, the 1 hr used at present is very safe.

On the other hand, pharmacists must consider the stability of their products and will not expose them to conditions greatly in excess of those needed to produce sterility. The B.P. recommends  $150^{\circ}\text{C}$  for 1 hr for oily solutions while the *International Pharmacopoeia* has the same temperature for oily solutions and suspensions but increases the time to 2 hours. There is no objection to the use of high temperatures where harmful effects cannot result, e.g. for glass vessels and containers, and for the sterilisation of these the *British Pharmacopoeia* specifies not less than 1 hr at not lower than  $160^{\circ}\text{C}$  while the U.S. and I.P.s recommend  $170^{\circ}\text{C}$  for 2 hr.

Finally, the very great heat resistance of the spores of certain soil organisms must be mentioned. Darmady *et al.* (1958) reported a dried soil that required 3 hr at  $150^{\circ}\text{C}$ , 90 min at  $160^{\circ}\text{C}$ , or 70 min at  $170^{\circ}\text{C}$  for sterilisation, and it can be seen that only the last of the methods given above would destroy bacteria of this resistance. Therefore, it is necessary to decide if it is essential for an acceptable process to kill, as well as pathogens, all saprophytes, whatever their source. Since, in

pharmaceutical work, drastic exposures could seriously affect the stability of preparations it seems reasonable to approve a method that has been shown, by carefully designed and frequent sterility tests on the products, to kill pathogens with a good margin of safety and to destroy the saprophytes that contaminate injections during preparation. The unlikely occurrence of a highly resistant saprophyte from an unusual source can be regarded as an acceptable risk.

## THE HOT AIR OVEN

### 1. Design

The designer of an oven for the sterilisation of pharmaceutical products must try to satisfy the following requirements—

- (a) Every article inside must receive the correct exposure, wherever it is placed.
- (b) The sterilising temperature must be reached quickly and maintained with little variation.

These aims are most nearly fulfilled by electrically-heated, thermostatically-controlled ovens with fan circulation.

Ovens of this type (Fig. 221) consist of an aluminium or stainless steel chamber separated from the outer case by a thick layer of glass fibre insulation. The hollow, flanged door is also filled with insulation and carries an asbestos gasket that provides a tight seal. The reflecting inner surfaces, the lagging and the gasket all help to prevent heat losses. In the best types the heaters are fixed to the outside

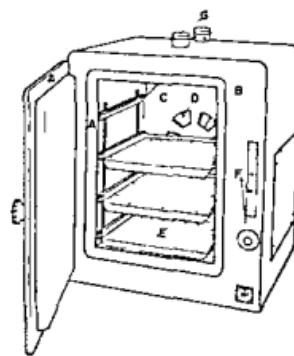


Fig. 221 HOT AIR OVEN

**A**—asbestos gasket  
**B**—outer case containing glass-fibre insulation, and heaters on chamber wall  
**C**—false wall  
**D**—fan  
**E**—perforated shelf  
**F**—regulator  
**G**—vents

(Courtesy Townson & Mercer Ltd.)

\* See page 272.

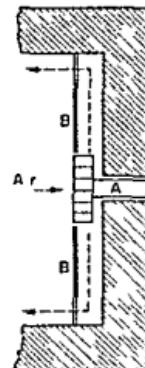
of the chamber, in positions chosen to prevent cool spots anywhere inside. Heat is transferred from the source to the articles in a hot air oven by conduction, convection and radiation. Conduction, along the shelves from the hot lining, cannot play a major part because of the limited pathways from the heat source and the frequently small area of contact (e.g. the bottom of a tin or bottle) between the article and the shelf. Convection is more important, especially in ovens with heaters restricted to beneath the floor, but air is a very poor heating agent because of its low specific heat, if it gives up a significant number of calories its temperature drops alarmingly and rapid return to the heat source is essential. C. Sykes (1958) illustrates this with some interesting figures. He points out that at 150°C and atmospheric pressure a 2 ft<sup>3</sup> oven contains about 65 G of air with a heat content of about 2 kcal more than at room temperature (20°C), a modest load for such an oven would be 3 kg and its temperature is unlikely to be appreciably affected by the small amount of heat in the air unless this can be given up and taken up quickly. Consequently, it is advantageous to supply the bulk of the heat by radiation, and this is the reason for arranging the heaters all round the chamber. Other essentials of the heater circuit of well-designed ovens are automatic boost heating to give minimum heating-up times, and accurate temperature control by easily-set regulators.

Even in a carefully loaded oven articles near the walls tend to screen those in the centre from radiation; therefore, it is important to make maximum use of the heating capacity of the hot air. To achieve this, its molecules must be encouraged to give up their heat energy quickly and to absorb more from the source as rapidly as possible. By circulating the air with a fan more of the air molecules are made to collide with the load (collision with other molecules being the only way in which they can pass on their energy) and with the hot chamber surfaces; in addition, pockets of stagnant cool air are dispersed. A common arrangement is to project the fan from the back of the chamber and put a baffle in front, the air is sucked through holes in the centre of the baffle, passed over the heated back wall and returned, towards the door, through openings at the corners of the baffle (Fig. 22.2). It is then sucked through the perforated shelves to the fan again. The chief improvement is a quicker rise in temperature of the load, also, there is slightly better uniformity of temperature, particularly near the door which, because it has no heaters, is cooler than other surfaces.

In a *loaded* oven the temperature variation (usually taken as the difference between the tem-

Fig. 22.2 SECTION OF REAR OF AN OVEN SHOWING AIR CIRCULATION PRODUCED BY A FAN

A—fan  
B—false back



perature at the centre and any other point, see B.S. 3421 1961) should not exceed 5°C once the sterilising temperature has been reached. For comparison, Dartmady and Brock (1954), using a gas heated oven with heaters in the bottom, and no fan, found a difference of 40°C between syringes at top and bottom. (See also Grainger and Smith, 1958.)

## 2. Method of Use

For the process to be reliable, every article, no matter where it is placed in the oven, must be at the correct temperature, throughout its mass, for the whole of the sterilisation period. Consequently, extra time must be allowed for the heat to penetrate the material and raise every part of it to the correct temperature. In some cases, such as large containers of poor conducting substances (e.g. powders) this is considerable. In a glass jar, 100 G of talc may take over half an hour longer than the oven air to reach 150°C. The most satisfactory procedure is to set up a preliminary experiment, in an oven loaded as for a normal process, with thermocouples in the centres of containers in different parts of the chamber, and one in the oven air. Then the difference between the times at which the air and the most slowly heated container reach the sterilising temperature is taken as the lag, and added to the sterilisation time in subsequent processes. To prevent overheating of more rapidly penetrated articles, only one type of material (powder, oil etc.) in one size and type of container, should be sterilised at one time and the lag should be determined previously for each.

An alternative, but less satisfactory method, is to immerse the bulb of a thermometer (usually fitted by a bung through a tubule in the top of the oven) at the centre of a container of the same size and content as those being sterilised. Then, timing of the

normal sterilisation period can begin as soon as the thermometer indicates the correct temperature. An objection to this procedure is that the middle of the top shelf is not the coolest part of the oven.

It is important to reduce the heating-up time to a minimum, partly for economy, but chiefly to prevent excessive overheating of the outer regions of materials and preparations during the time that heat is penetrating to their centres. The best way is to use small containers through which the heat will be transferred quickly even if the contents are poor conductors. An ounce is a wise upper limit for pharmaceutical substances such as powders and oils. The walls of the container should be as thin as practicable and of good heat-conducting material, e.g. metal rather than glass for powders. Tins should be dull or blackened to absorb and not reflect heat and, as a general rule, all containers should be either tall and narrow (e.g. a long cylindrical tin) or shallow and very wide (e.g. a Petri dish) so that heat can penetrate rapidly in one direction. Glassware must be cleaned thoroughly because heat transfer will be impaired if the surface is coated with a greasy or dirty film.

Because articles sterilised by dry heat are not often used immediately, precautions must be taken to ensure that they are sterile when used. Contamination may occur during cooling (because non-sterile air is drawn in as the oven air contracts), immediately the oven door is opened and during storage. Some form of packaging is necessary, and its nature depends on whether or not the outside of the article must remain uncontaminated. For example, glass pipettes (because they are dipped into sterile liquids) and hypodermic syringes and needles must be externally sterile and, therefore, they are completely wrapped in paper or packed in tubes of card, metal or glass. Items, such as glass vessels and containers of sterile products need protection at the mouth only and this can be given by a cotton-wool plug or metal cap (for vessels) or a screw cap with a suitable liner (for sterile preparations).

After the articles have been suitably packed, they are carefully arranged on the oven shelves, taking into account the following points—

1 They must be well spaced to interfere as little as possible with the air flow. Some of the shelf perforations should be left uncovered, and piling must be staggered. In an over-loaded oven heating up is very considerably delayed and heat distribution is very uneven.

2 As far as possible, the spacing should not obscure the centre items from wall radiation.

3 Contact with walls and floor must be prevented

because these are hotter than the oven air and may damage certain materials and other cotton-wool plugs and paper wrapping.

4 The packing of considerable numbers of comparatively small items, such as Petri dishes and graduated pipettes, in large tins should only be done with a full appreciation of the considerable delay in heating-up that results. The air inside cannot easily escape and acts as an efficient insulator of the contents. It is much better to wrap the pipettes individually and the dishes in twos or threes.

5 The screw caps of containers should be loosened half a turn to prevent distortion of the closure or bursting of the container from the expansion of the contents and the entrapped air.

Next the following are checked—

- 1 *The thermoregulator*. This is adjusted if necessary.
- 2 *The thermometer*. If this is not built into the oven it should be fitted, as already described, and must project as far as possible into the oven (e.g. 6 in.) to prevent a significant effect on the reading from the cold exposed stem (Wood and Tilley, 1945).
- 3 *The vents or vents* (on the top of the oven). To obtain the most accurate control of the temperature these should be closed.

The door can then be shut and the heaters and fan (if this is separately controlled) switched on. When the thermometer shows that the oven air has reached sterilisation temperature, heating is continued for the lag and exposure times.

After switching off, the door is left closed until the temperature has fallen considerably (ideally to about 40°C), thus prevents breakages. The bottle caps are tightened as soon as possible.

A hot-air oven should have a lock, otherwise it is too easy for the door to be opened in the middle of the process—Just to dry a bottle, for example.

Automatic control is often used in hospitals and industry, and this, and the checking of the process that is necessary at regular intervals, are discussed in the section on moist-heat sterilisation.

### 3. Applications

#### (a) GLASSWARE

Most of the glassware regularly sterilised by dry heat is required for aseptic or bacteriological techniques. It includes flasks, beakers, tubes, containers (e.g. ampoules), pipettes, petri dishes and all-glass syringes.

First, they must be thoroughly degreased by washing in hot water and detergent and rinsing well, followed by a final three rinses in pyrogenic distilled

water. New, or very dirty, articles should be soaked first in chromic cleaning solution overnight.

Then they are dried in a drying oven at about 65°C. The hot air oven shown in Fig. 221 can be used for this purpose by opening both the vents at the top. The fan sucks in fresh air through the one at the rear and draws it through a metal tube in the oven insulation. The resulting warm, dry air is passed into the chamber where it displaces some of the moisture-saturated air which can escape through the front vent. This design gives very rapid drying rates.

Flasks and beakers are plugged with long, fibre non absorbent cotton wool enclosed in two layers of muslin to reduce the chance of small fibres falling inside. The plugged mouths are then covered with a piece of brown paper held by string tied in a half bow that can be quickly undone by pulling one end with forceps. When it is particularly important to exclude all fibres, the paper cover is sufficient, provided the article is used soon after sterilisation.

For tubes, aluminium caps are a useful alternative to plugs and are much more convenient in aseptic technique because of the speed and ease with which they can be removed and replaced. Some conical flasks are designed to take these caps.

Graduated pipettes are plugged to about half an inch by inserting a small piece of cotton wool and cutting or burning off the projecting end. They may be rolled in brown paper, taking care to enclose the tip adequately and to twist the other end tightly. It is important not to have the twist at the tip because it is intended to indicate to the user the end that he can safely handle. An alternative method is to use card tubes, these are sealed with staples at one end and are plugged at the other. Often coloured cotton wool is used to distinguish different sizes.

Capillary (Pasteur) pipettes are used in large numbers in sterility testing and are most conveniently packed after plugging in groups of about 12 in a large glass tube. To prevent damage to the capillaries, packing is needed at the bottom of the tube, if cotton wool is used it should be enclosed in paper to stop fibres from clinging to the pipette tips.

The packing of petri dishes has been mentioned already, and all glass syringes are discussed in chapter 29.

Most rubbers cannot be sterilised by dry heat because they perish. Therefore, elastic bands must not be used for fixing paper covers. The only types of rubber liner satisfactory in a screw cap are silicone and some kinds of chloroprene.

The *British Pharmacopœia* suggests an exposure time of not less than 1 hour at not lower than 160°C., but for loads of this kind there can be no objection

to the more drastic conditions of some other pharmacopœias except, perhaps, that plugs and paper will show signs of charring.

#### (b) OTHER EQUIPMENT

The equipment for aseptic processing includes some articles of porcelain (such as mortars, pestles, evaporating basins and tiles) and metal (including beakers and dishes of stainless steel, scissors, scalpels and ointment tubes) that can be sterilised by dry heat.

It is most convenient to wrap these items completely, and in the case of eye-ointment tubes this avoids the use of plugs, the fibres from which are particularly objectionable in eye preparations. Pestles should be entirely porcelain, because wooden handles may be fixed with a cement that softens at sterilising temperatures and allows the parts to separate, the same problem exists with the older types of glass and metal syringe. Tube caps must be of a thermosetting plastic of adequate heat resistance.

For the sterilisation of cutting instruments the methods described in chapter 29 are preferable because long heating at high temperatures in hot air causes slight oxidation, which reduces the sharpness of the blades.

#### (c) OILS AND SIMILAR ANHYDROUS MATERIALS

Dry heat sterilisation is of particular value when contact with moisture must be avoided, e.g. for powders (considered in the next section), vehicles used for oily injections (e.g. fixed oils—see Coulthard (1935) and O'Brien and Parish (1935), ethyl oleate and other fatty acid esters), ingredients of ointment bases (e.g. liquid, soft and hard paraffins, wool fat, wool alcohols and beeswax) and medical lubricants (e.g. glycerin).

Apart from powders, these materials are fluid at sterilisation temperatures and, consequently, convection will assist their heating-up. Nevertheless, the lag times for all but the smallest containers are considerable, and large containers should be avoided, particularly since overheating can cause slight decomposition in some cases, e.g. vegetable oils.

If, as is normal, ointment base ingredients are to be used almost immediately for aseptic processing they can be sterilised in large petri dishes in layers of about  $\frac{1}{2}$  in depth, when they will heat up almost as quickly as the oven air. The most suitable final container for an ointment or similar material (e.g. soft paraffin, used as a lubricant) is a metal collapsible tube because, compared with a jar, there is less risk of contaminating the rest of the product when part is used. However, a tube cannot be sterilised

after filling because, if closed, it may burst from expansion of the contents, and, if left unsealed, with a temporary cover over the open end, the melted material may leak from the cap which, even if tight at first, may distort slightly during heating. It is necessary to fill the sterile preparation aseptically into the sterile tube, a rather difficult procedure to carry out satisfactorily. Therefore, for the convenience of sterilisation in the final container, a jar is sometimes used, but this must be small, thin walled, shallow and fitted with a metal cap and card liner, only a thin layer of the preparation should be included and, preferably, only sufficient for a single application because of the difficulty of avoiding contamination during use.

For fluids such as glycerin and liquid paraffin small screw-capped bottles of the McCartney type, but with heat resistant waxed-card instead of rubber liners, are suitable, a small size is preferable because it will heat up and be used quickly.

When a sterile oily solution or suspension is required, e.g. for depot medication, the method of preparation depends on the thermostability of the medicament. Some, e.g. procaine penicillin (used as a suspension in arachis or sesame oil, gelled with 2 per cent aluminium stearate) and propylidone (used as a suspension in arachis oil) are thermolabile, and the vehicle is sterilised separately, allowed to cool, the medicament incorporated aseptically and the preparation packed in a sterile injection container. Others, e.g. deoxycortone acetate, dimercaprol, oestradiol benzoate, progesterone, testosterone phenylpropionate and propionate, nandrolone phenylpropionate and phenol are sufficiently stable for the completed preparations to be sterilised by dry heat. When such solutions are packed in multi-dose containers, several difficulties arise, and these are discussed in chapter 23. Ampoules are much more satisfactory and, although, in some cases, the variable dose of the injection seems justification for multi-dose packing it should be possible to meet this problem by a range of volumes and strengths in ampoules. Ampoules are obligatory for dimercaprol injection because replacement of the container air by an inert gas is necessary.

For the sterilisation of oily injections and vehicles the B.P. requires exposure of the complete contents of each container to 150°C for 1 hr. The need to determine and allow for lag times is inherent in this requirement. It is surprising that the International Pharmacopoeia recommends a two-hour exposure at the same temperature.

Paraffin gauze dressing B.P.C. is often sterilised by dry heat. It consists of a fabric of special weave

(Cooper and Gunn, 1957) impregnated with yellow soft paraffin. Manufacturers supply single squares in plastic envelopes or many pieces, interleaved with a special paper, in a tin. When the material is extemporaneously prepared in hospitals the second method, but often without the paper, is used. The tins must be shallow, i.e. about 1 in., and if solder has been used in their construction it must not melt under sterilisation conditions. The gauze, in pieces or in a continuous length (large areas of burned tissue may be covered with it), is arranged about 20 layers deep inside the tin and covered with filtered soft paraffin heated to about 100°C. The final depth of material should be about  $\frac{1}{2}$  in. to avoid an excessive lag time which could cause overheating and damage to the outer part of the dressing. The tins should be arranged in the oven to present the maximum possible surface to the radiated heat and, after sterilisation, should be sealed with adhesive tape. The B.P.C. recommends 150°C for 1 hr and it is most important to appreciate that this refers to the material, and not the oven air, because the lag may be considerable. As the Codex also allows other suitable methods of sterilisation, a technique used in America and summarised in the *United States Pharmacopoeia* is interesting. The gauze is sterilised by the normal moist heat sterilisation method for dressings, this is less likely to damage the fibres and there is greater certainty of sterilisation because the dressing is not embedded in paraffin meanwhile. The paraffin is sterilised by dry heat, separately, and, after cooling to 100°C, is added aseptically to the gauze.

(See also Savage and Chambers (1942) and Gershenfeld, McClenahan and Yarlett (1954).)

#### (d) POWDERS

Broadly, dusting powders fall into two groups, medical and surgical. The main use of the former is the treatment of superficial skin conditions, and sterility is not often essential. Nevertheless, they must be free from dangerous pathogens and, since some of the mineral substances used as ingredients (e.g. talc and kaolin) are from the earth and may be contaminated with the spores of tetanus (*Clostridium tetani*), gas gangrene (*Clostridium welchii*) and anthrax (*Bacillus anthracis*), these should be sterilised by maintaining the whole of the powder at not less than 160°C for at least an hour. Purified talc is extensively used because of its excellent flow properties, it is an ingredient of several dusting powders of the *British Pharmaceutical Codex* and the *British National Formulary* and for this purpose must be sterile. Normally, the other ingredients are not sterilised and, therefore, there is no need to heat the

talc in a bacteria-proof container, a shallow metal tray, with a thin paper cover to prevent disturbance by the forced convection, is suitable, and, afterwards, the powder can be transferred, not necessarily aseptically, to a sterile or clean, dry bottle for storage until use. The rate of heat transfer through powders is very slow because of their low conductivity and the insulating effect of the air trapped between their particles, consequently, they must be sterilised in small amounts or, for bulk powders like talc, in thin layers (Bateson, Box and Gunn, 1958).

Surgical powders must be sterile because they are used in body cavities and major wounds, or on burns. The best container is a small envelope of heat-resistant paper, holding about 5 G, inside a slightly larger envelope. Heat penetration of this pack is rapid and the outer wrapper keeps the inner one sterile, therefore, when the latter is aseptically removed in the operating theatre or ward it will not contaminate the surgeon's or nurse's gloves, or the patient. An alternative pack is a glass vial, holding from 5 to 15 G, with a stainless steel sifter top protected by a metal cap. As the outside of the glass will become contaminated during storage and must be touched during use, it is less suitable for surgical purposes than a double envelope.

An important preparation commonly packed in double envelopes is Cord powder B P C. It contains alum, talc and zinc oxide and must be sterile because it is used to dust the cut umbilical cords of infants. The mixture of fine powders is dried at 150°C for 1 hr, cooled, repowdered to break down clumps, packed in envelopes holding about 5 G and sterilised by heating for a sufficient time to ensure that the whole of the powder is exposed to 150°C for 1 hour.

The following substances present special problems that complicate their sterilisation by dry heat—

#### *Starch*

Starch does not flow easily because its particles tend to stick together, and this is made worse if the moisture content is high. However, if it is dried at 100°C for about an hour and then powdered (with the other ingredients) before sterilisation (at 150°C for 1 hr) its flow properties are enhanced. It flows more freely, also, if about 1 per cent of light magnesium oxide is added, and an explanation of this has been suggested by Craik (1958). See also, Craik and Miller (1958).

#### *Sulphonamides*

Sulphonamides are no longer widely used topically, because sensitisation may occur and lead to rashes

and fevers. To prevent absorption of a toxic dose the amount applied is rarely greater than 10 G.

Because, most often, they are used as surgical dusting powders, diluents, such as talc, are not added since they may cause severe and even dangerous tissue reactions in body cavities. Sometimes, however, sulphonamides are required as diluents for other antibacterial drugs, e.g. amineacrine hydrochloride and penicillin. As penicillin is thermolabile, aseptic addition to the sterilised sulphonamide is necessary, the antibiotic being supplied sterile by the manufacturer.

Official sources give sterilisation methods for sulphadiazine, sulphadimidine, sulphanilamide and sulphathiazole.

The main problem has been to produce a free-flowing powder without discolouration, and Shotton and Simons (1950), using sulphanilamide, showed that a number of factors are important—

*1 The particle size* Powders finer than 100 mesh became electrified during grinding and would not flow freely.

*2 The moisture content* Powders with more than 0.5 per cent moisture became electrified less readily, powders with more than 0.25 per cent caked when heated in screw-capped bottles but powders with nearly 1 per cent did not cake in paper envelopes because the water vapour could escape.

*3 The recrystallisation solvent* Discolouration occurred if alcohol, but not if water, was used.

*4 The envelope paper* Some types were discoloured by, and others discoloured, the powder, a good vegetable parchment was best.

*5 Added substances* Zinc oxide and kaolin gave a more free-flowing powder (see Craik, 1958). However, their addition is undesirable because of the possibility of tissue reactions.

The usual method is to use crystals of suitable fineness, to dry these in a thin layer at 100°C, to pack (preferably in double paper envelopes) and then to sterilise by maintaining at 150°C for 1 hr.

#### *Lactose*

Occasionally this has been used as a diluent for penicillin. Since penicillin must be kept dry to avoid decomposition, the lactose should be dried in an oven at 105°C, then sterilised and, finally, mixed with the antibiotic. Paper envelopes do not give sufficient protection against moisture, and a well-closed sifter vial should be used.

Some powders, e.g. sodium chloride, will withstand heating to red heat in a muffle furnace (see the methods for the sterilisation of powdered substances

in the B.P.) In addition to killing micro-organisms this treatment will also destroy pyrogens and is sometimes used for this purpose (see chapter 23).

Other applications of dry-heat sterilisation include certain of the absorbable glove powders and the absorbable haemostatic, gelatin sponge.

#### Advantages of Dry heat Sterilisation

1 It can be used for substances that would be harmed by moisture, e.g. oily materials and powders.

2 Provided sufficient time for penetration is allowed, it is suitable for assembled equipment, e.g. all glass syringes. In moist heat sterilisation, steam or water must be in contact with every surface, and this is not always possible, e.g. on the closely-fitting adjacent surfaces of the barrel and plunger of an assembled syringe, especially if these have been lubricated with an oily fluid.

3 It is less damaging to glass and metal equipment than moist heat. Repeated exposure of glass to moisture at high temperatures can produce clouding and alkali extraction, while rusting is a serious risk when instruments are sterilised by wet methods.

#### Disadvantages of Dry-heat Sterilisation

1 The drastic conditions—high temperature, long exposure and very long heating up times. Most medicaments, rubbers and plastics are much too thermolabile for sterilisation by this process.

2 It is unsuitable for surgical dressings. The natural moisture of the fibres quickly vaporises and deterioration follows, this is first evidenced by discolouration and brittleness and, later, by charring. In addition, because dressings are usually large and their fibres are poor conductors of heat, heating up is extremely slow and is made worse by the large amount of insulating air that fills the interstices. The outside becomes badly overheated before the dressing is at sterilisation temperature throughout. The gauze in paraffin gauze is not exposed to such extreme conditions because the heating-up time is minimised by exposing a thin layer only and taking care not to entrap air when pouring on the paraffin. Also, the natural moisture is not lost as quickly as from a dressing in air. Nevertheless, it is not unusual for slight charring to occur and this is one reason why the American method was developed.

## MOIST HEAT

Micro-organisms can be exposed to moist heat by using hot water, boiling water, steam at atmospheric pressure (steaming) or steam under pressure (auto-claving).

#### SUSCEPTIBILITY OF MICRO-ORGANISMS

Moist heat is believed to destroy micro-organisms by causing protein coagulation or denaturation (Rahn, 1945). Published information shows clearly that it can kill micro-organisms at lower temperatures and in shorter times than dry heat. For example, all vegetative bacteria are destroyed by 1 hr at 80°C, and very few will survive 10 minutes at this temperature (1½ hr at 100°C). (The exposures in the brackets give the comparable figures for dry-heat sterilisation.) The spores of pathogens, however, require 30 min at 115°C (3 hr at 140°C). Mould spores are killed by ½ hr at 80°C (1½ hr at 115°C) which will also destroy, easily, the vegetative forms of moulds, yeasts, and yeast spores. As in the case of dry heat, most viruses are about as susceptible as vegetative bacteria, with only a few having a resistance nearer to that of spores, e.g. infantile diarrhoea and homologous serum jaundice.

Effective exposures are decided after careful study of the susceptibility to heat of many organisms, particularly the most heat resistant spore forming

pathogens. This susceptibility is most usefully expressed by the Thermal Death Time which is the shortest time necessary at a particular temperature to kill a population of specified size. If conditions such as the dimensions of the exposure tube, age of the culture and nature of the suspending medium are carefully controlled, comparable results can be obtained for different organisms. From the results for the most resistant spore-producing species (e.g. *Clostridium tetani*) safe heat treatments can be deduced by adding safety margins to the temperature, time or both. For example, a value given for the thermal death time of *Clostridium tetani* spores is 10 min at 105°C and, therefore, the exposure mentioned above, of 30 min at 115°C, can be used with confidence for sterilisation.

However, as with dry heat sterilisation, it is not possible to kill the spores of certain saprophytes with the exposures that will destroy all pathogens. This is illustrated in Table 22.1, which records some of the results of experiments carried out in 1920 by Bigelow and Esty on the spores of a thermophilic *Bacillus*. Notice particularly the time at 115°C and compare it with the figures given for *Clostridium tetani*.

The argument presented under 'Dry Heat', against basing sterilisation exposures on the resistances of

Table 221

Temperature °C	Minimum sterilising time
100	22 hr
115	84 min
120	23 "
130	3.5 "
140	1 "

non pathogens rarely met in pharmaceutical products, also applies in this case.

To produce 115°C under moist conditions requires steam under pressure and quite complicated equipment, 100°C would be much more convenient and would allow the use of very simple apparatus and boiling water or steam at atmospheric pressure. Unfortunately, this is impracticable because very long exposure at 100°C is needed to kill certain pathogenic spores, e.g. 3 hr for some strains of *Clostridium tetani* and *Clostridium welchii*. (As Table 221 shows, the spores of some thermophilic saprophytes require even longer.) A long exposure at a relatively low temperature may cause more damage to a pharmaceutical preparation than a shorter treatment at a higher temperature (Schou, 1950). This factor, as well as the inconvenience of a process taking several hours, explains why boiling and steaming are not used for the sterilisation of parenteral solutions.

#### FACTORS AFFECTING THE THERMAL DESTRUCTION OF MICRO ORGANISMS

##### 1 pH

The thermal resistance of most micro-organisms is highest within the pH range 6 to 8. The bactericidal effects of high acidity and alkalinity, mentioned in the discussion of factors affecting the growth of bacteria, are increased by temperature rise and, consequently, acid or alkaline solutions are easier to sterilise (Coulthard, 1939, Davis, 1934). Certain medicaments, e.g. alkaloidal salts, produce non-neutral solutions while adjustment or buffering to acid or, less commonly, alkaline pHs may be used for preserving stability. These conditions will assist the sterilisation of an injection.

##### 2 Inhibitory Medicaments

Solutions of some medicaments are harmful to bacteria (Davis, 1935, Todd and Smith, 1932). In certain cases, e.g. quinine hydrochloride, the effect is due partly to pH but in others, e.g. leptazol, the substance is directly toxic. Although some non-sporing bacteria, e.g. *Staphylococcus aureus*, are

killed in 24 hours at room temperature by a number of medicaments at injection strength, spores are rarely affected unless the solutions are heated under conditions of time and temperature approaching those at which the heat alone would sterilise. Consequently, very few injections can be safely regarded as self sterilising and the nearest that official sources come to recognising this potentiality is the permission to omit a bactericide from multi dose containers of leptazol and ethanolamine oleate injections. Nevertheless, the contribution of the medicament to the destruction of micro-organisms gives to the sterilisation of many injections additional safety that is not available when most other articles are being sterilised.

##### 3 Antibacterial Agents

The common bactericides are even more effective than most medicaments at assisting heat to destroy bacteria and this facilitates the sterilisation of multi dose injections.

Also, several of these injectable bactericides can, in higher concentrations, reduce the sterilising temperature for parenteral solutions from 115°C to 100°C. This is valuable for relatively heat sensitive substances and is utilised in the sterilisation method known as Heating with a Bactericide (q.v.).

##### 4 Protective Substances

Micro-organisms are less easily killed in media containing high concentrations of organic substances such as proteins and carbohydrates. The reasons are uncertain, but protein is believed to form a protective coat on the cells.

Since most protein-containing injections are thermolabile and cannot be heat sterilised the above effect is most likely to be found in preparations containing thermostable carbohydrates, e.g. dextrose, which is used in concentrations up to 50 per cent. However, experience shows that any increase in resistance of organisms in these solutions is adequately covered by normal sterilising exposures.

The problem is more important in the sterilisation of equipment such as containers, tubing and syringes. If these are carelessly cleaned after use for injections containing proteins (e.g. blood and its products, and immunological products) or carbohydrates (e.g. dextrose and dextran) organisms trapped in or below the deposits produced on drying will be protected from the steam and some may survive. Efficient cleaning techniques are essential.

##### 5 Initial Number of Organisms

When a population of living organisms is heated at a lethal temperature the cells are not all killed at

once, instead, the number of survivors gradually falls as the exposure is prolonged. If a certain fraction of the initial number is destroyed in a particular time interval then the same fraction of the survivors will be destroyed in the succeeding interval of equal duration and this will continue until the whole population has been sterilised, e.g. with an initial number of  $10^6$ /ml and a death rate of 90 per cent/min., the numbers surviving after each of the first six minutes will be  $10^5$ ,  $10^4$ ,  $10^3$ ,  $10^2$ , 10, and 1 per ml respectively. This is known as an exponential or logarithmic death rate, the numbers of survivors falling in geometric progression at equal time intervals. If the numbers of survivors (in unit volume) are plotted against time a smooth curve is obtained and if the logarithms of the numbers are used a straight line results, (see Fig. 22.3). Deviations from an exponential death rate are not uncommon, reference should be made to the work of Jordan, Jacobs and Davis (1947) for further information.

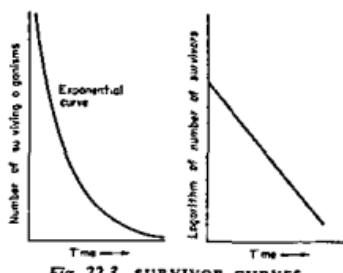


Fig. 22.3 SURVIVOR CURVES

From the figures provided it can be seen that if the initial concentration of organisms is small the time for sterilisation will be less. Therefore, by using well-cleaned equipment and containers and taking precautions to reduce the entry of bacteria to solutions during preparation, the sterilisation of injections can be facilitated.

### PRINCIPLES OF STERILISATION BY STEAM UNDER PRESSURE

Pressure itself has no sterilising power. Steam is used under pressure simply as a means of obtaining temperatures high enough to destroy micro-organisms quickly. For example, if the steriliser contains no air and the steam is not superheated (see later) the temperatures corresponding to particular gauge pressures are as shown in Table 22.2

Table 22.2

Steam pressure (lb/in. <sup>2</sup> g)	Approximately equivalent temperature (°C)
10	115
15	121
20	126
30	134

The abbreviation lb/in.<sup>2</sup>g means pounds per square inch gauge (as shown on the gauge of the steriliser), i.e. pressure above atmospheric. Absolute pressure (lb/in.<sup>2</sup>abs) is gauge pressure plus atmospheric pressure. Since atmospheric pressure is approximately 15 lb/in.<sup>2</sup>, 10 lb/in.<sup>2</sup>g is approximately equivalent to 25 lb/in.<sup>2</sup>abs, 15 lb/in.<sup>2</sup>g to 30 lb/in.<sup>2</sup>abs etc.

Steam for sterilisation can be obtained in two ways—

1 In portable sterilisers it is produced from water inside, and since during the process the steam is in constant contact with this water it carries suspended

droplets ('spray') and is known as *wet* saturated steam.

2 For large sterilisers used for surgical dressings and batches of intravenous fluids steam is generated in a separate boiler (often one that supplies steam generally to the institution) and piped to the apparatus. If precautions are taken, this steam carries practically no suspended water when it enters the steriliser and is called *dry* saturated steam.

Steam is described as saturated when it is at a temperature corresponding to the liquid boiling point appropriate to its pressure (Lyle, 1947). Two important properties of saturated steam are indicated by the phase diagram shown in Fig. 22.4.

The phase boundary on this diagram is obtained by joining points representing saturated steam temperatures at different pressures, e.g. 115°C at 10 lb/in.<sup>2</sup>g, 121°C at 15 lb/in.<sup>2</sup>g and 134°C at 30 lb/in.<sup>2</sup>g. Consider the saturated steam represented by A. However little this is cooled (e.g. from A to B) it will deposit water, provided the pressure remains constant. The same will happen if the pressure is raised without alteration of temperature (A to C) but this is not as important in sterilisation. Next, imagine that saturated steam, A, is isolated from water. If it is heated, without change of pressure (A to D), or the pressure is lowered, without change of temperature (A to E), the steam must become hotter because there is no water from which further evaporation can occur. Therefore, it is no

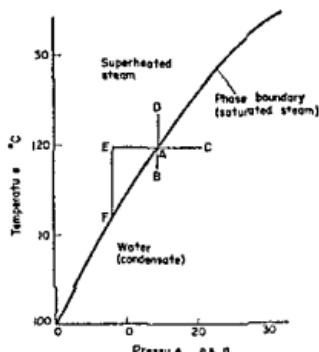


Fig. 22.4 PHASE DIAGRAM

longer at the temperature corresponding to the liquid boiling point appropriate to its pressure and is called *superheated steam*.

Very slight cooling will not make superheated steam condense. Before this can occur its temperature must be reduced to the corresponding saturated steam point (e.g. D to A or E to F).

#### SATURATED STEAM

Saturated steam is an efficient sterilising agent because—

*1 A Large Percentage of its Heat Energy is in the Form of Latent Heat.* The heat energy in steam is in two forms

(a) *Sensible heat.* The heat required to raise the temperature of water from freezing to boiling point

(b) *Latent heat.* The additional heat needed to convert water at its boiling point to steam at the same temperature

The amount of heat energy required to raise the temperature of 1 lb of water by  $1^{\circ}\text{F}$  is known as one British Thermal Unit (1 Btu)

Therefore, the sensible heat in 1 lb of steam is  $212 - 32 = 180 \text{ Btu}$

But the latent heat is much greater 971 Btu, i.e. about 84 per cent of the total heat energy

These values are for steam produced at  $100^{\circ}\text{C}$  ( $212^{\circ}\text{F}$ ). Table 22.3 shows the corresponding relationships at higher temperatures

The sensible heats are greater because of the higher boiling points. The latent heats are lower because at higher pressures the vapour molecules are pressed closer together and, therefore, need less

Table 22.3

Temperature °C	Temperature °F	Heat (Btu) Sensible	Heat (Btu) Latent	Latent to total (%)
115	239	208	953	82
121	250	218	946	81
126	259	227	940	80
134	274	243	930	79

energy to reach and maintain their positions in space. However, the percentage of 'latent' relative to 'total' remains high in each case.

When saturated steam condenses it liberates all its latent heat immediately. This happens in a steriliser whenever the steam touches the cool surface of an article inside. The large amount of latent heat is given to the article and makes a major contribution in raising it to sterilisation temperature. Moreover, since all the sensible heat is retained by the condensate there is no fall of temperature in the surroundings. It is easy to see why saturated steam is a much better heating agent than hot air, the heat content of the latter is small, while heat transfer is slow and accompanied by a drop in air temperature.

*2 It Condenses on Cooling.* The protein coagulation by which moist heat is believed to kill micro-organisms occurs at lower temperatures if plenty of moisture is available. A possible explanation was provided by Lewith (1890) who showed that the coagulation temperature of egg albumen depends on the amount of water present—

Water (%)	Coagulation Temp (°C)
50	56
25	77 ca
6	145

In the absence of water, oxidation occurs before appreciable coagulation takes place (see p. 309).

If, as this work suggests, the coagulating and, therefore, the lethal agent in steam sterilisation is *very hot water* the readiness of saturated steam to condense is a tremendous advantage. In addition, the importance of water may partly explain why spores are more resistant to sterilisation than vegetative bacteria (see p. 222).

Aqueous injections can be satisfactorily sterilised in sealed containers because the organisms inside are in contact with abundant water, but other articles, such as glassware, dressings and instruments, must be packed in a way that allows steam to reach and condense on every surface.

**3 When it Condenses it Contracts to an Extremely Small Volume** At 121°C only 1 ml of water is produced from the condensation of 865 ml of steam. In consequence, a region of low pressure is created into which more steam rapidly flows. This, in turn, condenses, gives up its latent heat and contracts, and the cycle is repeated until the article has been raised to steam temperature.

This property of saturated steam ensures quick penetration throughout bulky porous materials such as surgical dressings. The inferiority of hot air in this respect is strikingly illustrated in Table 22.4 which compares the heating up times for a roll of flannel.

Table 22.4

	<i>Hot air</i>	<i>Saturated steam</i>
Air temperature	150°C	Steam temperature 120°C
Temperature inside roll after 3 hr	80°C	Temperature inside roll after 10 min 117°C

To summarise, the advantages of saturated steam as a sterilising agent are—

- (a) It flows quickly to and, if required, into every article in the load (volume contraction)
- (b) It rapidly heats the load to sterilisation temperature (liberation of latent heat) Advantage (a) assists this
- (c) It provides, at high temperature, the moisture essential for killing micro-organisms (production of condensate)

The brackets contain the property on which each advantage depends

### SUPERHEATED STEAM

Superheated steam is no more efficient at transferring heat, penetrating a load and destroying micro-organisms than hot air at the same temperature.

Superheating is unlikely in portable sterilisers containing water because continued heating simply produces more saturated steam. It would occur if the steriliser boiled dry, but this is improbable if the apparatus is used properly.

In apparatus fed by boiler steam superheating can take place in several ways—

**1 If the Jacket Temperature is Higher than the Chamber Temperature** Usually the body of a large steriliser is surrounded by a steam jacket to prevent

excessive condensation on the inner surface of the chamber. This condensate would soak dressings and considerably prolong the drying stage at the end of the process.

If the jacket steam is hotter than the steam in the chamber heat is conducted across the intervening wall and the chamber steam becomes superheated. Therefore, the jacket and chamber should be at the same temperature. Before the danger was appreciated a 10°C difference was common.

**2 If Air is Mixed with Steam in the Chamber** Dalton's law of partial pressures states that if two or more gases are contained in a closed vessel the pressure exerted by the mixture is the sum of the pressures that each would exert when occupying the same volume alone. Consequently, if, at 15 lb/in.<sup>2</sup>g., the chamber contains 10 per cent of air this will contribute 1.5 lb/in.<sup>2</sup>g. of the total pressure and only 13.5 lb/in.<sup>2</sup>g. will be due to the steam. Fig. 22.4 shows that steam of this pressure is saturated only at 118°C and, therefore, will be superheated if the chamber is at the temperature corresponding to 15 lb/in.<sup>2</sup>g. of saturated steam, i.e. 121°C. This superheated steam will not liberate its latent heat until it has been cooled 3°C. This type of superheating is very difficult to detect because neither the thermometer nor the pressure gauge, separately or together, will reveal it. Therefore, air must be removed as completely as possible from a steriliser heated by steam alone.

**3 As the Steam Passes through the Reducing Valve** The pressure of the steam supply from a hospital or works boiler is usually much greater than the pressure required for sterilisation. It may be as much as 80 lb/in.<sup>2</sup>g. and must be passed through a reducing valve before admission to the steriliser. The heat content is unaffected by this operation and is greater than saturated steam can hold at the lower pressure. Consequently, the steam becomes superheated. In fact, this rarely happens in practice, for the following reasons—

(a) Often the steam arriving at the valve contains suspended water droplets and the excess heat is completely absorbed by the evaporation of these. Sometimes the supply steam pressure is adjusted so that the degree of reduction will dry most of the wetness out of the steam without superheating it. For example, if the steam supply is 55 lb/in.<sup>2</sup>g. and contains 6 per cent of suspended water, the latter is reduced to 2 per cent after reduction to 15 lb/in.<sup>2</sup>g. (Bowie, 1955).

(b) Usually the reducing valve is connected to the steriliser by an unlagged pipe. Unless this is very

short, the superheat is lost to the cool surrounding atmosphere as the steam passes through.

A further method of superheating is important when dressings or fabrics are being sterilised—

*4 By the Heat Produced when Cellulosic Fibre Absorb Moisture* Cotton and other cellulosic fibres can absorb a considerable quantity of moisture, about 20 per cent of their dry weight. The absorption process, in which the water becomes bound to the fibre structure, is accompanied by the evolution of heat. If the water is obtained from dry saturated steam a much larger amount of latent heat is liberated too. The total heat generated from these two sources is called the heat of absorption and is responsible for the rapid heating up of porous fibrous materials in a correctly operated steriliser.

However, if, before sterilisation, the materials have a very low moisture content, absorption of moisture and evolution of heat continue after the steam temperature has been reached and, consequently, the fibres and nearby steam become superheated. The relationship between the initial moisture content of the fibres and the degree of superheating has been predicted by Henry (1959) and found approximately correct in practice by Knox, Penikett and Duncan (1960)—Table 22.5

Table 22.5

Initial moisture content (%)	Max. amount of Superheating at 20 lb/in <sup>2</sup> g (°C)
Less than 1	9
About 5	1 to 2
More than 8	negligible

This superheating applies only to the fibres and the steam adjacent to them, e.g. within the drum or wrapper containing the dressings. The rest of the steam in the steriliser is saturated and, very slowly, the superheated fibres come into equilibrium with this by conduction. However, this takes many hours and the superheated state exists for the whole of a normal sterilisation cycle.

Correctly stored fabrics and dressings contain about 5 per cent of moisture, and the few degrees of superheating can be tolerated (see below). Storage under very dry conditions can lead to excessive superheating and, therefore, prevent sterilisation.

There is one way in which a very large amount of superheating can result from the absorption of water by fibres. If a steriliser containing dressings is pre-

heated by the jacket for some time before steam is admitted the fibres become very hot as well as very dry. When steam is introduced, the heat of absorption is added to the heat already in the fibres and considerable superheating takes place, it may be 50°C or more. Walter (1948), who drew attention to this danger, reported that the superheating sometimes caused dressings to burst into flames when the chamber door was opened.

Until recently, fabric loads were often preheated because of the wrong belief that excessive wetting, necessitating long drying, would occur if steam was admitted while the contents were cold. In fact, the main cause of soaked dressings is wet steam (qv). Although several hours of preheating are necessary to produce the startling degrees of superheating just quoted, the practice is dangerous and cannot be approved.

Nowadays it is usual to remove all or part of the air from a dressings steriliser by vacuum before the steam is admitted. Meanwhile, steam is in the jacket and some preheating will take place. However, Knox *et al.* (1960) have shown that in the few minutes required for evacuation the amount of drying is insignificant.

#### TOLEERABLE DEGREES OF SUPERHEATING

Because of its importance as a provider of water for the lethal hydrolysis of microbial cell constituents, steam would not be expected to be a satisfactory sterilising agent while it is superheated. However, Savage (1937) disproved this assumption by showing that 5 to 15, or even more, degrees of superheat, depending on the saturation temperature before superheating, can be tolerated before the steam becomes ineffective. His experiments were carried out in a special apparatus in which spores were exposed to a current of steam superheated to various degrees. A short exposure time of 10 minutes was chosen to ensure that sterilisation did not take place in every case. The general pattern of his results is shown in Fig. 22.5.

From this it can be seen that unless the initial steam temperature is low a few degrees of superheat do not prevent sterilisation. For example, although sterility cannot be obtained if the initial temperature is 105°C, over 15°C of superheat are necessary before the steam becomes inefficient if it is at 115°C initially. To understand these results it must be appreciated that steam produced from a solution is superheated in respect to water because it is evolved at a higher temperature than steam from pure water. Consequently, steam superheated in respect to water can be in equilibrium with a suitable solution.

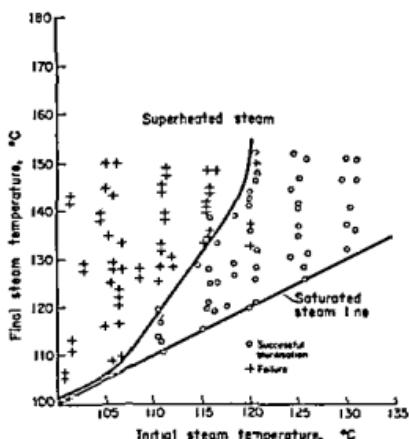


Fig. 22.5 RELATIONSHIP BETWEEN STERILISING EFFICIENCY AND AMOUNT OF SUPERHEATING

Bacteria and their spores contain solutions of cell constituents and these will be in equilibrium with and take up water from slightly superheated steam which is, therefore, an effective sterilising agent. Savage suggests that superheated steam from saturated steam at low initial steam temperatures is ineffective because at the pressures corresponding to these temperatures the cells will contain less water and, therefore, be less easily killed.

Although this work provided the valuable information that a few degrees of superheating are not disastrous at the temperatures normally used for sterilisation, it was not intended to encourage the use of superheated steam. Today, brief exposures such as 3 min at 135°C are popular and any adverse effect of superheating on sterilising efficiency is more likely to show than when a longer time is used. The most sensible procedure is to prevent superheating in every possible way and to set the tolerable maximum low, e.g. 5°C. In the B.P. method of sterilisation by heating in an autoclave exposure to saturated steam is required.

#### PRESENCE OF AIR

##### I IN PORTABLE STERILISERS

Pure saturated steam has a definite temperature at a particular pressure but a mixture of air and steam at the same pressure has a lower temperature because the air makes a much smaller contribution to the sensible heat than steam of equivalent pressure. This

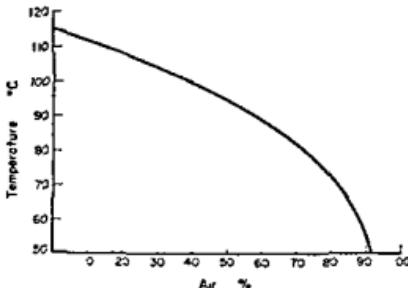


Fig. 22.6 TEMPERATURES OF STEAM ADULTERATED WITH AIR (10 LB/in<sup>2</sup>G)

is illustrated in Fig. 22.6 from which the figures in Table 22.6 have been obtained.

In some types of portable steriliser the pressure gauge is the only indicator of the conditions inside, but its readings cannot be translated into equivalent saturated steam temperatures unless all the air has been expelled. Only then is it safe to assume, for example, that 10 lb/in.<sup>2</sup>g is equivalent to 115°C.

Table 22.6

Air (%)	Temp (°C) at 10 lb/in <sup>2</sup> g
0	115.0
50	95.0
90	56.7

However, as would be expected, if the steam in the mixture is raised to the correct temperature it becomes a perfectly satisfactory sterilising agent in spite of the presence of the air. Application of the extra heat required cannot superheat the steam because there is plenty of water in this type of steriliser. Any superheated steam would be desuperheated quickly by evaporation of some of the water. The temperature increase is accompanied by a rise in pressure but the gauge cannot be used because its reading depends on the amount of air present, which will not be known. However, if the steriliser can be fitted with a thermometer, as is possible with some types, air removal becomes unnecessary.

Similar considerations apply to the sterilisation of aqueous fluids in bottles or ampoules. Micro-organisms on inner surfaces above the liquid level are exposed to a steam (produced in this case from the contained water) and air mixture, but since this will be raised to the correct temperature by conduction through the walls from the steam outside,

satisfactory conditions for sterilisation will exist. If there is less or no air in the external steam the contents of the container will be at a higher pressure and this is an important reason for using good quality glass and taking care when cooling and opening the steriliser.

## 2 IN LARGE STERILISERS FED BY BOILER STEAM

In this type of equipment contamination of the steam with air may take place in several ways—

(a) After closing the door, the air in the chamber is sucked out by vacuum-producing equipment and/or pushed out by the incoming steam. Inefficient performance of these procedures will leave air inside.

(b) Unless a very high vacuum is drawn some air remains in the interstices of porous loads. This may be completely trapped when steam is admitted or gradually pushed into the chamber atmosphere during the exposure period. Also, if tubes (containing syringes etc.) or drums (of dressings) are packed upright in the steriliser, residual air may be forced to their bottoms and be unable to escape.

(c) Boiler steam always contains a small amount of air (under 5 per cent).

Dilution of steam with air has more complicated and, often, more serious effects than in a portable steriliser. These include—

(i) *Reduced Temperature at the Required Pressure*  
Table 22.7 shows the temperatures of mixtures of steam and air at equilibrium in a large steriliser from which different amounts of air have been removed by vacuum producing equipment.

At first glance these temperatures may seem inconsistent with those given previously for air steam mixtures at 10 lb/in<sup>2</sup>g. Then it was stated, for example, that saturated steam/air mixture containing 50 per cent of each has a temperature of 95°C. In Table 22.7 the figures show a temperature of 105°C if 50 per cent of the air has been removed. The explanation is that in the first case the air is assumed to contribute 50 per cent of the pressure at 10 lb/in<sup>2</sup>g, while in the second it provides 50 per cent of atmospheric pressure—

### 1st case

$$10 \text{ lb/in}^2\text{g} \equiv 25 \text{ lb/in}^2\text{abs}$$

Therefore, contribution of the steam, which provides the heat, =  $\frac{25}{2} = 12.5 \text{ lb/in}^2\text{abs}$

Steam tables show that this is equivalent to 95°C.

### 2nd case

$$\text{Atmospheric pressure} = 30 \text{ in Hg} \equiv 15 \text{ lb/in}^2\text{abs}$$

$$\text{Therefore, a } 15 \text{ in vacuum will leave } \frac{15}{2} = 7.5 \text{ lb/in}^2\text{abs}$$

lb/in<sup>2</sup>abs of air in the chamber. Consequently, at 10 lb/in<sup>2</sup>g (25 lb/in<sup>2</sup>abs) the steam contribution =  $25 - 7.5 = 17.5 \text{ lb/in}^2\text{abs}$ . This is equivalent to 105°C.

(ii) *Danger of Superheating* It is not possible to produce satisfactory sterilising conditions, as in a portable steriliser, by heating an air steam mixture to the correct temperature, because in a steriliser containing no water the steam would become superheated. (See also 'Superheated Steam').

(iii) *Delayed or Obstructed Steam Penetration* In a portable steriliser the turbulence produced by the boiling water intimately mixes the unexpelled air with the steam. This facilitates heat transfer from the steam to the air and the latter quickly reaches steam temperature.

In a large steriliser there is little turbulence after the initial admission of steam and, as a result, mixture of the air and steam takes place very slowly. A uniform temperature is reached eventually, but a long time is required, particularly if the steriliser is heavily loaded with porous materials. Meanwhile, the chamber contains pockets and layers of air, much cooler than the steam, in which articles do not receive a sterilising exposure. Pockets will be mainly at the centre of porous materials or in the bottom of containers with small or narrow openings (e.g. a plugged tube and a tin with a loosely fitting lid, respectively). Even if these are correctly positioned in the steriliser (see 'Loading') it may be difficult for the air to escape. For example, the air will penetrate from all directions into a dressing wrapped in paper or fabric, and residual air will be pushed to the middle and trapped there. Even if this absorbs

Table 22.7

Fraction of air removed	All	$\frac{1}{2}(20 \text{ in vac})$	$\frac{1}{2}(15 \text{ in vac})$	$\frac{1}{2}(10 \text{ in vac})$	Nil
Temperature (°C) at 10 lb/in <sup>2</sup> g	115	109	105	100	90

sufficient heat from the steam to reach the correct temperature the absence of moisture within the pocket will make sterilisation very unlikely.

If a large amount of air is present it collects as a layer at the bottom of the chamber because it is approximately twice as heavy as steam at the same temperature. This layer is increased by contact of the air/steam mixture with the cool (unjacketed) door, where the steam condenses, leaving behind the air which sinks down the door to the floor of the steriliser (Savage, 1959). In one investigation the temperature in this layer was only 100°C although the steam above was at 121°C, understandably, articles at the bottom of the apparatus remained unsterile.

In the vertical type of large steriliser occasionally used for infusion fluids air is usually expelled by steam admitted from the top, and unless displacement is prolonged some may be left between the bottles and form dangerous pockets of insulation.

A film of air clings by molecular attraction to every article in a steriliser. This must be kept as thin as possible because it greatly reduces the rate of heat transfer. Still air is a worse conductor than the best lagging, and a thickness of only 1/100 in offers the same resistance to heat transfer as 11 ft of copper (Lyle, 1947). Air from an air/steam mixture collects at any surface on which the steam condenses, and although in a portable steriliser it will be dispersed by the turbulence, in a large steriliser it may remain and seriously delay heating-up.

For these reasons, the removal of air is the most important aim in the design and operation of modern large sterilisers.

#### WETNESS OF THE STEAM SUPPLY

Steam reaching a large steriliser is rarely dry-saturated. If it comes from an inefficient boiler it may be carrying droplets of water, but even if almost-dry steam passes into the supply pipes and these are well lagged, some condensation will occur en route to the steriliser. The steam may arrive carrying 10 to 20 per cent by weight of water. This represents a serious loss of valuable heat and, in addition, the admission of wet steam is harmful when fabrics and dressings are being sterilised. The water soaks the outsides of the materials and delays the penetration of steam and displacement of air. Longer drying is required at the end of the process and this increases the risk of damaging the fibres, particularly since a hot jacket, unnecessary when the steam is dry (Penikett, Rowe and Robson, 1958), must be used.

A pool of condensate that has not properly

drained out through the discharge channel at the bottom of the chamber is another source of wetness. It can be prevented, if necessary, by slightly raising the end of the steriliser distal to the channel.

Wetness in the supply steam is removed by a separator and the reducing valve. A dryness fraction better than 0.95 is desirable.

(For further information on the principles of steam sterilisation see Bowie (1955), Perkins (1956) and Savage (1954, 1959).)

#### Exposure Times in Saturated Steam

Thermal death times for the spores of all pathogens and many thermophiles and soil bacteria have been published. Although the results of different workers are not always comparable, because the experimental conditions were not exactly the same, it is possible from the accumulated data to predict the minimum sterilising exposure at one or more temperatures. Approximately equivalent exposures at other temperatures can then be calculated from the fact that the death rate of spores increases 8 to 10 times for each 10°C rise in temperature over the range 100 to 135°C (Rahn, 1945). In this way Table 22.8, which shows equivalent sterilising exposures, was obtained by Thiel, Burton and McClemon (1952).

Table 22.8

Temperature (°C)	Time
100	20 hr
110	150 min
115	51 min
121	15 min
125	6.5 min
130	2.5 min

When compared with these figures the exposure of 30 min at 115 to 116°C used in the autoclaving method of the B.P. seems inadequate but long and satisfactory experience has confirmed its safety. Some possible explanations have been given in the discussion of the factors affecting the thermal destruction of micro-organisms.

A Medical Research Council working party on

Table 22.9

Temperature (°C)	Time (min)
121	15
126	10
134	3

pressure steam sterilisers (M R C Report, 1959), with the sterilisation of dressings and fabrics particularly in mind, recommended the three exposures in Table 229.

Until recently the temperature used for dressings sterilisation was 121°C or, less often, 126°C. Modern

sterilisers usually operate at about 134°C and offer the attractive advantage of a considerable saving in time.

A sufficient allowance for heating the load to sterilisation temperature must be added to all exposure times.

## DESIGN AND OPERATION OF STEAM STERILISERS

The apparatus for sterilisation by steam under pressure is called an autoclave or steam steriliser. Usually, the portable types used for small scale production of injection solutions are cylindrical and upright, they may be less than a foot in internal diameter and depth. The types previously referred to as large sterilisers are almost always fixed (e.g. to a steam supply) and are generally horizontal and either cylindrical or rectangular. The internal dimensions of the cylindrical type may be up to 2 ft 6 in in diameter and 4 ft in length while rectangular forms can be as large as 4 ft 4 in high, 3 ft 3 in wide and 4 ft 4 in long internally.

### A PORTABLE STERILISERS

The two types of portable steriliser are most simply described as pressure controlled and temperature-controlled.

In a pressure-controlled type the pressure gauge is the sole indicator of the internal conditions and, therefore, all the air must be removed before the sterilising exposure begins.

In the temperature controlled type a thermometer or thermostat is used to indicate or ensure respectively that the exposure temperature has been reached and it is not essential to expel the air.

#### 1 Pressure-controlled

An example is the 'Portable' autoclave (Fig. 227).

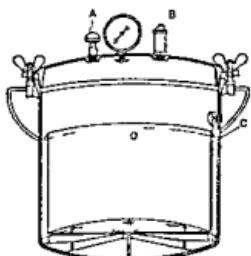


Fig. 227 THE 'PORTABLE' AUTOCLAVE  
A—vent B—adjustable safety valve C—syphon tube  
(Courtesy Arnold & Sons (Bastilledon) Ltd.)

#### (a) DESIGN

This autoclave has a strong cylindrical body made of an aluminium alloy and provided with a bucket type handle. Inside, on the bottom, are several radial ribs on which rests a light removable inner chamber. Around the rim are eight bolts that swing up into slots on the lid where they are held in position by wing nuts. The curved lid, concave on the side exposed to the steam pressure, carries in a recessed housing a rubber gasket that gives a steam-tight seal with the body. On the lid are three controls, a vent through which air is expelled, a pressure gauge that records steam pressure and vacuum, and a safety valve. At the top of this valve is a knurled nut that can be raised or lowered to decrease or increase the tension in a spring holding a ball bearing over a hole in the lid. With the nut raised an internal pressure of 10 lb/in<sup>2</sup> will open the valve and release the steam, but when the nut has been lowered 15 lb/in<sup>2</sup> is required. The standard model is heated on a gas-ring, but an electrically heated model is available.

Inside the steriliser is a tube leading from the bottom to a tap on the outside, and this adaptation is said to make the apparatus suitable for the sterilisation of dressings and gloves. If the tap is opened over a sink or other large receiver immediately after the sterilising exposure the internal pressure expels the water and most of the steam, leaving only the moisture absorbed during the process to be dried out of the materials. This is done by closing the tap again and leaving the steriliser to cool, when the resulting vacuum extracts much of the remaining wetness. Study of the theoretical principles underlying the successful sterilisation of porous loads will disclose several objections to the use of a steriliser of this type for this purpose (except, perhaps, in an emergency) and no further reference will be made to the application.

#### (b) OPERATION

The gas ring should be on a sheet of asbestos or other insulating material to protect the bench from heat radiated from the bottom of the autoclave. It is necessary to be particularly careful with plastic

surfaces because they may be lifted and distorted by the heat.

The siphon tap must be tightly closed or water will gush out when pressure develops inside.

Water is put in to the level of the bottom of the inner chamber and the articles for sterilisation are loosely arranged in the latter. Tight packing is avoided to leave room for expansion and prevent breakages, also air cannot escape easily from the narrow pockets between closely adjacent containers. Caps of bottled fluids should be screwed down tightly, there is no danger of explosion because the internal pressures are approximately balanced by the steam pressure outside.

The lid is put in position, taking care that all the hinged bolts will slip easily into the slots. Then the wing nuts are lightly turned and afterwards tightened in opposite pairs. If some of the bolts are not in place when tightening begins the lid may twist, and then it is impossible to get them into position. Also, if all the nuts on one side are tightened first it may not be possible to get a steam tight seal on the other.

The vent is opened and the safety valve is set at the required pressure, which, for the *British Pharmacopoeia* injections, is 10 lb/in.<sup>2</sup>g.

Heating is started over a full gas. Steam is allowed to issue freely from the vent for 5 minutes, which in a portable autoclave is long enough to expel the air. Timing of this must not start when the first trickle of steam appears, vigorous emission for the whole time is necessary.

The vent is closed, and when the pointer of the gauge reaches 10 lb/in.<sup>2</sup>g timing of the exposure begins. It is advisable to record immediately, and in a conspicuous place, the beginning and end of this period, this will prevent accidental under- or over-exposure. At the required pressure the safety valve opens and noisily releases excess steam. The flame is now turned down to a height that maintains the pressure but minimises steam release. If the valve is allowed to blow vigorously throughout the exposure period the noise and the steamy atmosphere are objectionable and, more important, the steriliser may boil dry. If the latter occurs, the steam becomes superheated, solutions in plugged or loosely capped containers are concentrated, and sealed bottles and ampoules may burst.

The B.P. requires exposure of the whole of the contents to 115 to 116°C for 30 min and, therefore, allowance must be made for the lag time. This should be predetermined, using thermocouples, but if, *in an emergency*, the information is not available a useful general rule is to add 10 min for containers of 101 to 250 ml, 15 min for 251 to 500 ml and 20 min

for 501 to 1,000 ml. Small and large volumes should not be sterilised in the same load because the correct time for the latter might cause overheating of medicaments in the former.

On completion of the exposure the gas is turned off and the pressure allowed to fall to atmospheric. Then the vent can be opened. This should not be done while the internal pressure is high because its sudden release would cause vigorous boiling and frothing over of liquids in unsealed containers and might lead to bursting of sealed bottles and ampoules. Opening the vent should not be delayed when atmospheric pressure has been reached because the vacuum produced by further cooling causes partial evaporation of solutions in loosely closed containers.

If the steriliser contains large, sealed bottles of fluids the lid should not be removed as soon as the vent has been opened. Large volumes cool slowly and their internal pressure will still be well above atmospheric. Explosions can occur if the bottles are stressed further by contact with the cool outside air. In some hospitals the staff wear fencing masks when removing infusion fluids from large sterilisers if unloading is essential while the contents are hot. The danger is slight with a portable steriliser if about 10 minutes cooling is allowed after opening the vent.

The instruction to load the steriliser before the water is heated should be modified if solutions, such as culture media, in plugged or loosely capped containers are being sterilised. If these are put into a cold autoclave their temperature follows closely that of the heating water and may be at boiling point during venting. Consequently, steam is produced from them, and concentration results. They should be put in when the water is boiling and allowance made for the increased lag that will result.

## 2. Temperature-controlled

The T.R.F. autoclave (Fig. 22.8) is an example.

### (a) DESIGN

The body is of stainless steel and fitted with opposite handles for carrying. A pair of semicircular plates or a wire basket with legs is used inside. The basket is necessary to hold wrapped equipment well above the water and prevent soaking of the wrapping. The plates increase the effective depth of the autoclave and are used for bottled fluids of large volume.

The rim is incurved and, being wider on one side, makes an oval opening. Two controls are fixed to the wide part. One is a short mercury in-glass thermometer inside a protective metal tube that is open at the bottom and over the range of scale from 100 to 125°C. The other is a thermostat through

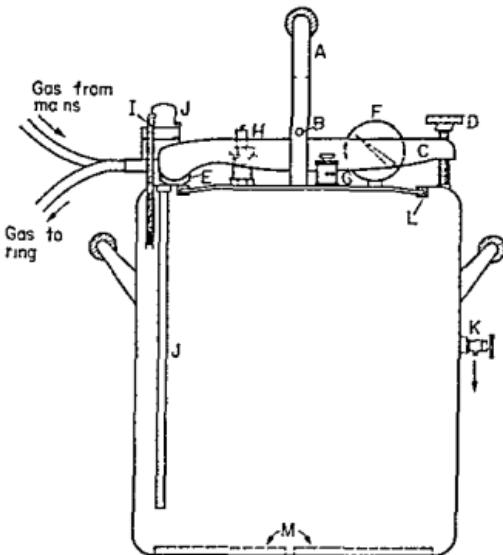


Fig 228 T R F AUTOCLAVE  
(DIAGRAMMATIC)

(Courtesy Taylor Rustless Fittings Co. Ltd.)

A Handle of lid	I Thermometer
B Rivet	J Thermmostat
C Crossbar	K Air vent
D Thumbscrew	L Gasket
E Pad	M Semicircular plates
F Pressure gauge	
G Safety valve	

H Whistling regulating valve

which the gas passes on its way to the ring, a bimetallic sensing element passes down almost to water level and operates a valve that controls the gas flow. This thermostat is set to keep the autoclave at the required temperature.

The air vent is on the side and discharges downwards to reduce danger to the operator if it is accidentally opened at full pressure.

The oval lid fits inside the body. Its edge is sheathed in a rubber gasket to give a steam tight joint with the underside of the rim. Its handle has two metal supports through which passes a strong rivet. Between the supports and under the rivet a crossbar can be fitted, this has a pad at one end and a thumbscrew at the other. The pad rests on the wide part of the rim and the thumbscrew fits into a small depression on the opposite side. By turning the screw the bar can be raised to pull the lid into position.

The lid also carries several controls—

- 1 A pressure gauge
- 2 A spring loaded safety valve of special design. This is set to blow off at 20 lb/in<sup>2</sup>g to prevent operation at higher pressures. The autoclave is tested at 35 lb/in<sup>2</sup>g but pressure apparatus is always treated with great respect and used well within the test limit.
- 3 An adjustable whistling pressure regulating

valve. In principle this resembles the safety valve of the Portable autoclave and it can be set to release steam at a predetermined pressure, e.g. 10 or 15 lb/in<sup>2</sup>g. The issuing steam passes through a whistle and gives warning that the required pressure has been reached, when it can then be screwed down to seal the valve. It is useful if the steriliser has to be left during heating up but often it is easy to watch the thermometer or pressure gauge at frequent intervals and, since these valves are a refinement rather than a necessity and require regular maintenance to keep them functioning accurately, some people prefer to ignore them.

Both gas and electrically heated models are available. The use of the former will be described

#### (b) OPERATION

First the thermostat is set by following the manufacturer's instructions. These, though simple, are detailed and need not be given here. On subsequent occasions the thermometer will show if adjustment is necessary.

Next, about three pints of water are put into the apparatus and the load carefully arranged on the plates or in the basket.

The lid is fitted in the following way. The autoclave is placed broad rim towards the operator, and

the lid held above with its long axis at right angles to the long axis of the opening and the pressure gauge to the far side. In this position its handle should be parallel with the handles on the body. The near edge is slipped under the broad rim keeping the lid as flat as possible and turning it anti-clockwise until it slides into position. The crossbar is passed through the handle and, after positioning the lid so that its edge is evenly covered by the rim all round, the thumbscrew is tightened to draw the lid firmly against the body. Anxious and unsuccessful struggles to perform this operation are sometimes seen. They can be avoided by starting with the handles parallel and the gauge on the far side, and taking care not to tilt the lid too steeply.

Since the thermostat has been adjusted to ensure the correct temperature it is not essential to vent the air. Timing begins when the thermometer shows 115 to 116°C.

Even in this type of autoclave there are advantages in expelling the air. First, it facilitates removal of air pockets from and between pieces of equipment. Secondly, it is possible to use the pressure gauge as an extra control, and a dial pressure gauge is easier to read than a mercury in glass thermometer, particularly from a distance. The T.R.F. autoclave replaced a type known as the Sphinx. The two are essentially similar in design and the Sphinx is likely to be used in schools, laboratories and dispensaries for many years to come. The major difference is that the thermometer is fitted into the lid of the Sphinx. In this position it is easily broken when the lid is put down after removal. Therefore, it is often left off and the gland sealed with a solid nut. The pressure gauge must then be used instead, and if the air is not vented timing cannot begin until the needle becomes steady, at a point that cannot be pre-determined because it depends on the amount of air inside. With venting, timing can start at the much more convenient point—when the pointer reaches 10 lb/in<sup>2</sup>g.

The remaining procedure is the same as for the Portable except in one respect. The T.R.F. pressure gauge cannot read vacuum. It has zero at one end, and if the vent is not opened soon after the internal pressure has fallen to atmospheric the vacuum caused by further cooling will strain the pointer and make the gauge inaccurate.

#### MAIN ADVANTAGES OF THE TWO TYPES

##### 1 Pressure controlled

- (a) The venting and the constant escape of steam during exposure ensure the absence of air

- (b) The lid is easier to fit and the method of fitting doesn't reduce the effective depth of the autoclave. When loading the T.R.F. or Sphinx space must be left for manoeuvring the lid.
- (c) The pressure regulator is simple and requires less attention than a thermostat.

##### 2 Temperature controlled

- (a) The internal temperature is controlled and shown.
- (b) The chief material of construction is stainless steel. Alkaline solutions will attack the aluminium alloy of the Portable.
- (c) The method of closure is safer because the lid cannot be removed while steam is at pressure inside. Additional safety is provided by the downward discharge of the vent.
- (d) Assuming the autoclave is not vented, liquids in loosely sealed containers are less likely to be concentrated.
- (e) The wire basket allows easy air drainage and is more satisfactory than a solid chamber in which there is the possibility of air layering or pocketing.

#### B LARGE STERILISERS

These are of complicated design (see, for example, B.S. 3219 1960 and 3220 1960) and there are so many types and modifications that the detailed description of any one is of limited value. They will be treated generally.

It is convenient to divide them into two classes, surgical-dressings sterilisers and sterilisers for bottled fluids. The former will be discussed first because most improvements in design have come from work on dressings sterilisation.

(See Bowie (1955, 1959, 1961), Howie (1961), Knox, Penkett and Duncan (1960), M.R.C. Report (1959), Penkett, Rowe and Robson (1958), Perkins (1956), Savage (1954), Scott (1957) and Walter (1948).)

##### 1. Surgical-dressings Sterilisers

###### OUTLINE OF THE PROCESS

The following stages are involved in the sterilisation of surgical dressings—

- (i) Suitably packed dressings are correctly loaded into the chamber.
- (ii) The door is closed and steam admitted to the jacket.
- (iii) Air is partially or almost completely removed by vacuum.
- (iv) Dry saturated steam is admitted and if necessary, may be used to displace the rest of the air.

- (v) Heating-up and exposure are carried out; air (drained from the dressings) and condensate are automatically discharged meanwhile
- (vi) The supply steam is cut off and the chamber vented
- (vii) The dressings are dried either by drawing a high vacuum or by using a partial vacuum to suck warm sterile air through them
- (viii) When high vacuum drying has been used the vacuum is broken by admitting sterile air

The above summary will make the next section easier to follow

#### DESIGN FEATURES

Dressings sterilisers are horizontal and have the shapes and sizes mentioned previously. They may be free-standing or built into a wall, the latter often have a door at each end to allow loading from one side and unloading, into a 'clean' area reserved for sterile materials, from the other. The doors are hinged, and close by a locking mechanism that prevents opening while the steam is at pressure inside. The chamber, apart from the door(s) is surrounded by a steam jacket which, in turn, is covered with lagging inside a metal casing. Within the chamber is a perforated shelf to keep articles from the bottom where they might become soaked by condensate.

#### *Removal of Air by Downward Displacement*

The air can be pushed out by the steam, a technique known as downward or gravity displacement, or sucked out by vacuum. In downward displacement, by admitting the steam to the top of the chamber the air is encouraged, because of its greater density, to sink to the bottom where it escapes through a discharge channel. To get an adequate discharge rate this channel must have a wide bore.

It is preferable to admit the steam near to the chamber roof but if, as in some older sterilisers, it comes in at the centre of the end a baffle is necessary to direct the flow upwards. These methods prevent the trapping of air pockets at the top (see Fig. 22.9)

A thermometer in the discharge channel is used to follow the process. While a mixture of air and steam is being vented the reading is lower than the temperature corresponding to the steam pressure.

After about 15 minutes the air will have been discharged from the chamber atmosphere and the parts of the load that the steam can penetrate easily. Since at this stage the thermometer reading will be at or near to the temperature corresponding to the gauge pressure it might appear safe to begin the

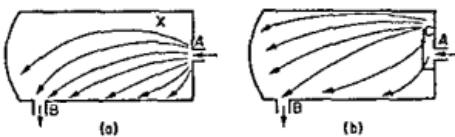


Fig. 22.9 ILLUSTRATION OF THE VALUE OF A BAFFLE

(a) Without (b) With  
A—steam inlet B—discharge channel C—baffle  
Without a baffle steam can rush directly to the outlet leaving air pockets (X) at the top

heating-up stage. However, the residual air in the load has still to be displaced, and this may take some time, particularly if the chamber and individual containers have been tightly packed or if drums without vents have been used and loaded upright. Complete removal of air from the latter can take over an hour. Even if displacement-retarding practices are avoided some air will escape from the packs rather slowly and must not be allowed to collect in the chamber.

The best method of clearance is to leave the discharge channel fully or partly open throughout the heating-up and exposure periods (compare the operation of the Portable Autoclave) but this wastes expensive steam, and normal practice is to include in the channel a valve that will open to vent air but close quickly afterwards. The balanced pressure type of thermostatic steam trap (Fig. 22.10) most closely fulfils this requirement. It contains a flexible bellows-like capsule filled with a mixture of water and a volatile liquid that boils at a lower temperature than water. The expansion of the mixture on heating stretches the bellows and gradually moves the poppet

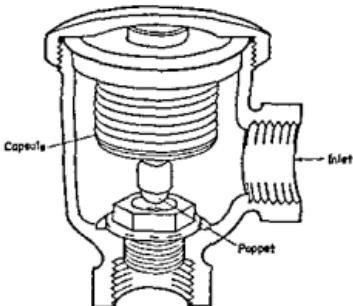


Fig. 22.10 BALANCED PRESSURE THERMOSTATIC TRAP

(Courtesy Spirax Sarco Ltd.)

towards its seat. At 2 to 3 degrees below the saturation temperature corresponding to the working pressure of the steriliser the mixture boils. This creates an internal vapour pressure which, just before saturation temperature is reached, is sufficiently above the external steam pressure to completely and tightly close the valve. It opens again when the internal pressure is reduced to below the pressure outside by contact with anything cooler than the boiling point of the mixture. Consequently, it will vent air, air steam mixtures and condensate and remain open until pure saturated steam reaches it again. The thermostatic traps used on sterilisers are called 'near to-steam' traps because they open within 3°C and close within 2°C of the saturated steam temperature. This occurs whatever the working temperature of the steriliser because if the steam pressure is decreased or increased the boiling point of the trap mixture, which is controlled by the pressure outside the bellows, is altered accordingly.

(For information on steam traps see Northcroft (1952) and the technical literature of Spirax Ltd.)

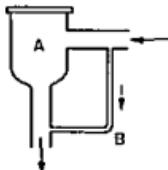


Fig. 22.11 BY PASS ROUND TRAP  
A—Trap B—By-pass

Unfortunately, small amounts of air do not lower the steam temperature enough to operate the trap, and it is advisable to have a small by pass, either a hole or a narrow pipe (Fig. 22.11) through which a slight but constant flow of steam can flush away the air without lowering the chamber pressure (Barson *et al.*, 1958). This modification has another use, because the trap is operated by temperature change it will not vent superheated steam or an air-superheated steam mixture that is at or above its closing temperature. Also, the bellows may be seriously strained or even burst by the high internal pressure caused by contact with steam that is greatly superheated. The flow through the by pass prevents the accumulation of superheated steam in the trap and also ensures its escape from the chamber.

Thermostatic traps only function satisfactorily if kept free from solid matter, and a strainer (Fig. 22.12) is essential in the channel on the steriliser side of the trap to collect pipe scale, broken glass, fragments of dressings and other debris. It must be emptied

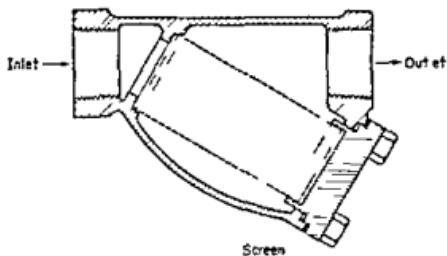


Fig. 22.12 STRAINER  
(Courtesy Spirax-Sarco Ltd.)

regularly. The air-break (see 'Removal of Condensate') gives visible indication that the trap is working properly.

To summarise, successful air removal by downward displacement requires—

- Loosely filled, steam permeable containers, placed and spaced in the steriliser to facilitate air discharge
- A strainer protected, near to-steam trap with a by pass in a wide discharge channel.

Under these conditions—

- Most of the air will be vented quickly and the rest prevented from accumulating in the chamber
- Heating up may begin when the channel temperature corresponds to the chamber gauge pressure

#### *Removal of Air by Vacuum*

- Low vacuum

Most older sterilisers have a steam ejector (Fig. 22.13), which is a venturi device operating in the

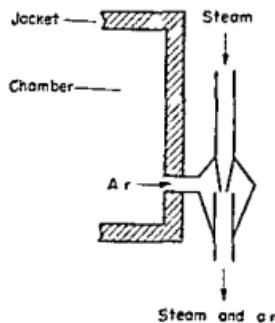


Fig. 22.13 STEAM EJECTOR

same way as a water pump but with steam instead of water. The vigorous discharge of steam from the inner pipe sucks air from the chamber. The maximum vacuum that can be drawn by this method is equivalent to 20 in Hg but it cannot always be achieved, especially when the supply steam pressure is low because of extra demands for other purposes such as winter heating. Only 15 in or less may be obtained even after a long period of evacuation, meanwhile steam is being wasted and the dressings are receiving prolonged and destructive heat treatment from the jacket.

Since, at best, only two thirds of the air can be removed, this method must not be used alone. Normal procedure is to evacuate, admit steam to just above atmospheric pressure and evacuate again, when the second vacuum should give a further two-thirds reduction of the remaining air. Satisfactory removal is obtained if this double vacuum technique is used in a steriliser provided with the discharge channel system described above or in one with an unmodified channel that is allowed to vent freely throughout the exposure.

#### (b) High vacuum

Potentially, vacuum is more efficient than downward displacement for removing air from a porous load. In downward displacement the entering steam impedes the escape of air, particularly when the containers are metal drums with no vent holes. In the double-vacuum method a similar problem exists, the residual air from the first evacuation will have been drawn towards the surface of the packs but the subsequent admission of steam will push it back to the centre again, and the second vacuum may still not suck it right out of the containers. However, with sufficiently high vacuum most of the air can be removed in one stage, even from tightly packed containers in a heavily loaded chamber. This method is used in modern high-temperature dressings sterilisers.

In a load of fabrics it is the air clinging to the fibre surfaces and trapped in the fine interstices that is most difficult to remove. In a sufficiently high vacuum the water of hydration in the fibres vaporises and this vapour pushes the air away. The M.R.C. Report (1959) states that at 15°C the pressure of water vapour in equilibrium with cotton containing 4 per cent by weight of water (the minimum likely in properly stored fabrics in this country) is about 5 mm Hg. Therefore, in theory, the chamber pressure must be reduced to this level before vaporisation will occur, but early work indicated that 20 mm Hg was adequate in practice, and this became the standard

for high vacuum sterilisers. A powerful vacuum pump that can reach this vacuum in about 5 minutes is required.

[Degree of vacuum may be expressed in two ways, as pressure remaining (absolute pressure) or pressure removed. The latter method is used above for low vacua, e.g. 20 in. Hg, and the values can be converted to absolute pressures, if required, by subtraction from atmospheric pressure (30 in Hg), e.g. 20 in Hg (removed) is equivalent to 10 in Hg abs (remaining). However, as atmospheric pressure is not always 30 in Hg, considerable errors could occur if the small absolute pressures resulting from high vacua were determined in this way and, consequently, they are measured directly, giving values such as 0.8 in (20 mm) Hg.]

Since high vacuum removes practically all the air there is virtually no hindrance to steam penetration, which takes place almost instantaneously. Heating-up is complete in a few seconds and the first stages of the sterilisation cycle (evacuation and heating up) shortened considerably—to about 6 min compared with over 20 min for an efficient downward displacement steriliser. When the heating-up time is long the sterilising temperature cannot be raised to obtain the advantage of a shorter exposure because the exterior of the dressings would be seriously damaged while heat was penetrating to the centre. The use of high vacuum removes this difficulty and allows the use of 135°C (32 lb/in<sup>2</sup>g) at which an exposure of only 3 minutes is adequate.

Nevertheless, Wilkinson and Peacock (1961a) have reported delays in reaching steam temperature in the lower part of dressings drums and have suggested retained air as the cause. The problem was solved by evacuating, as usual, to 20 mm abs, then admitting steam to 200 mm and, finally, re-evacuating to 20. The steam warms and moistens the fibres and when the second vacuum is drawn this extra moisture 'boils out' (more readily than the moisture present originally because of its higher temperature) and helps to displace the air.

Bowie (1961), using large packs, was also unable to obtain instantaneous penetration of steam. He recommended that pumping should not stop when 20 mm has been reached but, instead, a vacuumstat should start a timer that would prolong the vacuum period to 8 to 10 min. At the end of this time the absolute pressure in the chamber is 4 to 6 mm Hg, much closer to the theoretical requirement stated in the M.R.C. Report. Although the cycle time is lengthened, instantaneous penetration and heating-up is ensured.

High vacuum presents special problems (Shotton

(1959), Train (1960) and Wilkinson (1959, 1960)) Valves and gaskets must be very carefully checked for vacuum tightness since this is more difficult to achieve than steam tightness. The conventional pressure gauge cannot register accurately both 45 lb/in.<sup>2</sup>abs (30 lb/in.<sup>2</sup>g) and 6 oz/in.<sup>2</sup>abs (20 mm Hg abs) and a special vacuum gauge is necessary. The pump must remove a large volume of air quickly, and the most satisfactory types are oil-sealed rotary and water ring pumps. The former draw a better vacuum but precautions are necessary to protect and free the oil from condensate when steam is being removed. Condensate is not a problem with water pumps but conventional types must be modified to obtain an adequate vacuum.

#### Drying the Supply Steam

The steam is first passed through a well lagged separator (Fig 22.14) in which suspended condensate is collected on baffles. (Compare the still heads used in the preparation of Water for Injection.) Then its pressure is lowered to sterilisation level by passage through a reducing valve and this dries it further (*see Superheating*). Modern sterilisers often have two valves, one providing 32 lb/in.<sup>2</sup>g for dressings, fabrics, instruments and utensils, and the other 17 lb/in.<sup>2</sup>g for rubber gloves and bottled fluids. The valves require a pressure gauge on each side.

It is difficult to prove that dry saturated steam is being supplied to a steriliser. Several devices have

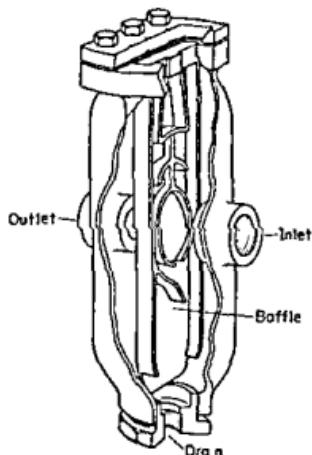


Fig 22.14 SEPARATOR  
(Courtesy Spirax-Sarco Ltd)

been developed (Gunn, 1961) but none is sufficiently reliable.

Jacket and chamber are supplied with steam at the same pressure. Sometimes the chamber is fed through the jacket but a separate supply line has the advantage of not passing the steam through a region in which considerable condensation is taking place.

#### Removal of Condensate

Rapid escape of condensate is necessary to prevent excessive wetting of the steam and consequent soaking of porous loads.

Condensation from contact of the steam with a cold load and chamber is reduced by having steam in the jacket during the preceding evaporation. Nevertheless, a considerable amount of condensate is produced on the cold door and by further heating of metal drums and other non-absorbent articles in the chamber. Also, if air can pocket in the jacket a cool, condensate-producing region of the chamber results, this is prevented by fitting an automatic air vent (a balanced pressure thermostatic steam trap) at the top of the jacket as far as possible from the steam inlet.

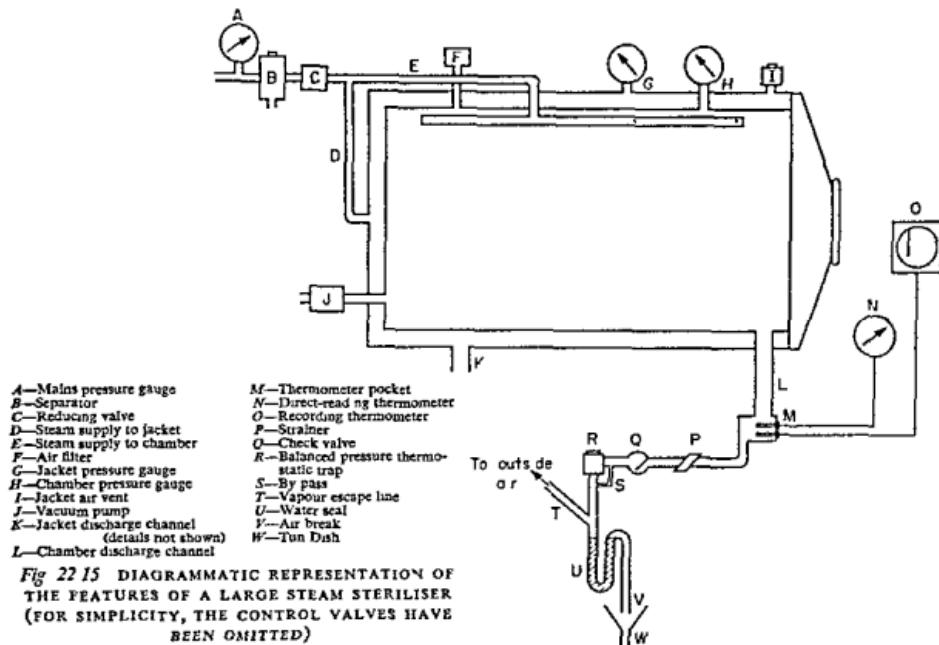
Condensate runs down to the bottom of the chamber and drains out through the same discharge channel as the air. The opening of this is near to the front of the steriliser, and rapid escape of condensate is ensured by a slope from the back. Alternatively, an extra drain may be fitted near the back. Between the steriliser and the trap there is about 2 ft of unlagged pipe in which the condensate cools sufficiently to operate the thermostatic valve.

In addition to the items already mentioned under removal of air, the channel usually contains the following, most of which are concerned with condensate discharge—

1 A check valve, at *Q* in Fig 22.15, between the trap and steriliser. This may be compared to a hung trapdoor, pressure on one side, from escaping steam, condensate or air, opens it, but pressure on the other closes it tightly. It prevents contamination of the chamber contents by unsterile air or water sucked back during the final vacuum.

2 A vapour escape line, shown at *T*, Fig 22.15, near to the end of the channel. This is an upwardly directed branch pipe through which steam and air escape.

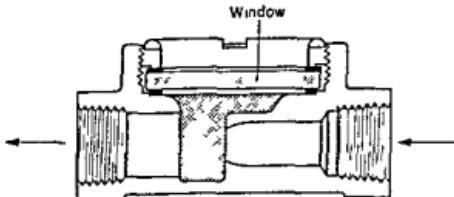
3 A water-seal at *U*, and air-break at *V*, in Fig 22.15, at the end of the channel. The arrival of condensate makes the seal overflow and escaping drops can be seen at the air-break and confirm that satisfactory discharge is occurring. The break must be in a conspicuous position.



4 A sight glass (Fig. 2216) on the outlet side of the trap. This is sometimes included, particularly on older sterilisers that have no visible air-break, to verify that the trap is working properly. It is a glass-windowed chamber in which the pipe is divided to show the flow of condensate.

5 A tun-dish, *W* in Fig. 2215, the funnel shaped waste that collects the condensate from the air break.

A somewhat simplified discharge channel is fitted to the jacket



**Fig. 2216 SIGHT GLASS**  
(Courtesy Spira-Sarco Ltd.)

#### Sensing the temperature

Because of the delay in reaching uniform temperature throughout a loaded large steriliser the pressure gauge cannot be used as the sole indicator of the internal conditions. A temperature-sensing device is essential, but this must not be at the top since an atmosphere of pure steam will be obtained there quickly. There are two possibilities. The first is to house the bulb of a mercury-in-steel thermometer in the discharge channel, but in a well drained recess so that the effluent flows freely around it (Fig. 2215). The second is to sense the temperature from the load by inserting thermocouples (Wilkinson and Peacock, 1962) into the centre and bottom of one or more control packs. Normal practice has been to use the drain thermometer because it is simpler to fit and less easy to damage, but recently Bowie (1961) and Harris and Allison (1961) have produced convincing evidence for sensing the temperature of high vacuum sterilisers from the load. Modern apparatus provides for both methods. Preliminary experiments with thermocouples are very useful for finding the

lags for different loads, after the drain has reached sterilisation temperature.

#### Drying the load

At the end of the exposure period porous loads are wet from absorbed condensate and must be dried. Until the introduction of a high vacuum technique three methods were in use—

(a) *By Radiation from the Jacket* The supply steam was shut off from the chamber but maintained at full pressure in the jacket. The chamber steam was allowed to vent to atmospheric pressure. The door was opened a fraction of an inch to allow cool air from the room to enter at the bottom as steam and warm air escaped from the top. Drying was considered satisfactory after about 20 minutes. There was a considerable risk of the unsterile air contaminating the wet dressings (compare the unsuitability of wet absorbent cotton wool plugs as tube seals) and although the outer layers of the packs were dry—but sometimes damaged by overheating—the centres remained damp.

(b) *By Sucking Warm, Filtered Air through the Load* After venting the chamber steam, as above, the venturi device was used to draw through the load air that had been filtered by passage through non-absorbent cotton wool and warmed to 50° to 60°C in a coiled pipe surrounded by steam. Moisture was entrained from the dressings and, with the aid of the hot jacket, drying was completed in approximately 20 minutes but, again, the insides were often left damp.

(c) *By Low Vacuum* The venturi was used, as in the previous method, but instead of admitting air, the maximum vacuum was drawn. Satisfactory drying can be achieved if the supply steam is dry, the condensate discharge efficient and a vacuum of 20 in Hg obtained. It should not be necessary to hold the vacuum (Penikett, Rowe and Robson, 1958). Poor results followed the use of wet steam and inadequate vacuum and, often, instead of correcting these faults, long holding times with steam in the jacket were adopted, with consequent damage to the outside of the load.

*The High vacuum Method.* The successful use of high vacuum for air removal soon led to a study of its suitability for drying.

If the wetness of dressings is entirely due to condensate from heating up it should be possible to remove it almost as quickly as it was absorbed if the heating up procedure is reversed, i.e. if the steam is drawn out and the pressure reduced back to 20 mm Hg abs. This was shown experimentally by Penikett,

Rowe and Robson (1958) and theoretically by Henry (1959). After evacuation to 50 mm Hg abs with one of the high efficiency pumps used for the initial vacuum the moisture content is only slightly greater than before sterilisation and there is a corresponding cooling of the load because the latent heat that raised it to sterilisation temperature has been used to vaporise the condensate. There is no thermal damage because the process only takes two or three minutes (the time to reach full vacuum) and the jacket need not be hot.

The method is not successful if the dressings are wet initially or become soaked by wet steam or inadequately drained condensate during exposure. In these circumstances the necessary additional latent heat of vaporisation has to be supplied from a hot jacket, a slow and damaging procedure.

#### Air Filtration

The air used for entrainment-drying or for breaking the vacuum after vacuum-drying must be sterile. Filtration through non-absorbent cotton wool has been the conventional method and is quite satisfactory if certain precautions are taken (Rice, 1958).

(i) *The Wool must be Non absorbent* The atmosphere of a steriliser room is moist, damp absorbent wool will let bacteria pass but impede the entry of air.

(ii) *It must be Sterile* Unsterile cotton wool, because of its method of manufacture, is heavily loaded with micro-organisms and can seriously contaminate the air.

(iii) *It must be Changed Regularly* Replacement should take place at least once a day and, preferably, every time the steriliser is used. Often in the past this requirement has been grossly ignored (Howie and Timbury, 1956).

(iv) *It must be Packed Carefully in the Filter Chamber* Usually it is enclosed in a small cylindrical canister with a pepper-pot lid. Packing should be tight enough to retain bacteria without seriously restricting air flow, special care is needed at the edge to leave no channels to function as by-passes. Readymade packs of suitable size are available, these can be slipped quickly and aseptically into the holder and when compressed by release of the chamber vacuum are of the correct density (Rice, 1960).

Recently, satisfactory filters have been developed from glass fibre paper (q.v.). In one type the sheets are supported between perforated metal plates in a flat cylindrical canister (Fig. 22.17). They have a long life, about a year, which can be further prolonged by using a prefilter of cotton wool as well.

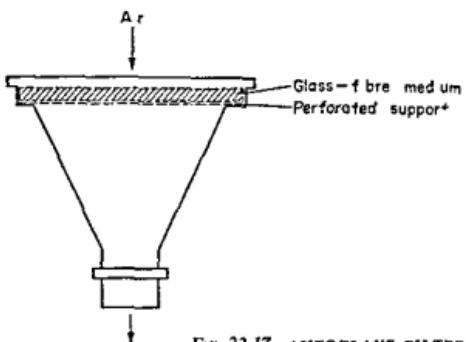


Fig. 2217 AUTOCLAVE FILTER  
(Courtesy Fokes Ltd.)

Efficiency tests are necessary at intervals (Rice, 1958). Sometimes porous ceramic filters are used

## 2. Sterilisers for Bottled Fluids

The sterilisation of bottled fluids is less complicated than dressings sterilisation but not in respects that allow much simplification of steriliser design. In fact, in attempts to solve one major problem—post sterilisation cooling of the load—extra features have been introduced

### OUTLINE OF THE PROCESS

The following stages are involved and should be compared with those for dressings sterilisation—

- (i) The bottles are loaded correctly
- (ii) The door is closed
- (iii) In some modern equipment, air is removed by high vacuum
- (iv) Dry saturated steam is admitted and in older, and some modern, sterilisers is used to displace the air
- (v) Heating-up and exposure are carried out, air and condensate being discharged meanwhile
- (vi) The supply steam is cut off
- (vii) Either (a) The chamber steam is allowed to vent slowly to reduce the internal pressure to atmospheric. Then the door is left closed until the load has cooled (a considerable time), or (b) A fine mist of cold water is sprayed over the bottles to cool them to safely below 100°C when they can be removed immediately

### DESIGN FEATURES

There are still some vertical cylindrical sterilisers in use for bottled fluids. They are a little easier to load and unload than their horizontal counterparts and fuller use can be made of their internal capacity

provided that more than one layer is included. However, the more recent horizontal rectangular type is particularly well suited to accommodate several layers of bottles without waste of space and is likely to become the most popular type in the future, the larger sizes hold several hundred half or one litre bottles and can be provided with a loading carriage on to which the whole internal shelf unit can be withdrawn.

An occasional burst bottle is unavoidable and, therefore, the internal surfaces must resist the corrosive action of salines. Stainless steel is used for the perforated trays or shelves while the other surfaces, if not entirely of the same material, are clad with it or nickel.

A jacket is often provided but is not essential. Condensate from the unjacketed (but lagged) chamber wall will not harm the load but since it represents a considerable heat loss a slight delay in heating up will result.

### Removal of Air by Downward Displacement

This method can be used with confidence because, generally, it is necessary to expel only the air surrounding the containers. The shelves should be well perforated and the load arranged loosely, to prevent residual air pockets between the articles.

Wilkinson and Peacock (1961b), using a rectangular steriliser containing over 200 1-litre bottles in four layers and heated by steam introduced at the top, found that the lowest layer took almost half an hour longer than the uppermost one to reach chamber temperature. Apparently, on admission, the steam stratifies above the air but exerts very little downward pressure because it condenses and contracts as it heats the upper layer. As the condensation rate decreases it penetrates deeper into the chamber but its advance is continually retarded by contact with fresh cold and condensate-producing surfaces. Therefore, expulsion of the air and heating of the lower part of the load are slow. Wilkinson and Peacock solved this problem by admitting steam through inlets designed to produce considerable turbulence in the steam air mixture. The steam reached all the bottles quickly and the temperature variation between the layers disappeared. The best distributor was an upwardly directed fish tail nozzle at the centre back of the steriliser but horizontal pipes with holes at intervals, near to the roof, were almost as good.

In some sterilisers for bottled fluids there is a modification to facilitate downward displacement. Below the thermometer chamber is a full bore bypass around the trap through which the air can be

vented until the drain temperature corresponds to the chamber pressure, then the by-pass is closed and the normal channel used. This procedure permits more rapid air escape than is possible through the narrow opening of the thermostatic valve. The larger types of steriliser have a drain outlet at both front and back.

#### *Removal of Air by High Vacuum*

Because of their high cost modern sterilisers are often designed so that by preselection of an appropriate cycle they can be used for either dressings or fluids. Since an efficient pump is provided for dressings it is convenient to use high vacuum instead of downward displacement for removing the air from loads of bottled fluids.

High vacuum has two advantages for porous loads, rapid and near-complete evacuation and almost immediate heating-up when steam is subsequently admitted. The second advantage does not apply to bottled fluids because the rate of heating up is limited by the rate of conduction through the container wall. Heating is taking place during downward displacement, but in the high vacuum method it does not begin until evacuation is complete, therefore, much of the time saved through quicker air removal by vacuum is lost through delay in heating up.

Breakages are more likely when high vacuum is

used because the steam immediately pours on to the whole of a cold load. In downward displacement, without high turbulence, the lower layers gradually become warm as the steam front approaches while with turbulence the lower temperature of the initial mixture of air and steam reduces the degree of thermal shock. In addition, with high vacuum, the containers and, particularly, the closures must be able to withstand the large pressure changes.

#### *Drying the Supply Steam*

Although witness has no adverse effect on loads of bottled fluids, the usual precautions are taken to provide dry saturated steam, because of its superior heat content.

#### *Removal of Condensate*

During the heating-up of bottled fluids a much greater amount of condensate is produced than in the same stage of dressings sterilisation. This is because a load of dressings has a smaller heat capacity and absorbs the condensate liberated on its fibres while the hot jacket reduces the quantity formed on the chamber wall.

Large volumes of condensate are not vented satisfactorily by balanced pressure traps, and modified ball float taps (Fig. 22.18) are used instead. As condensate collects in the trap chamber the float rises and opens the valve, the steam pressure

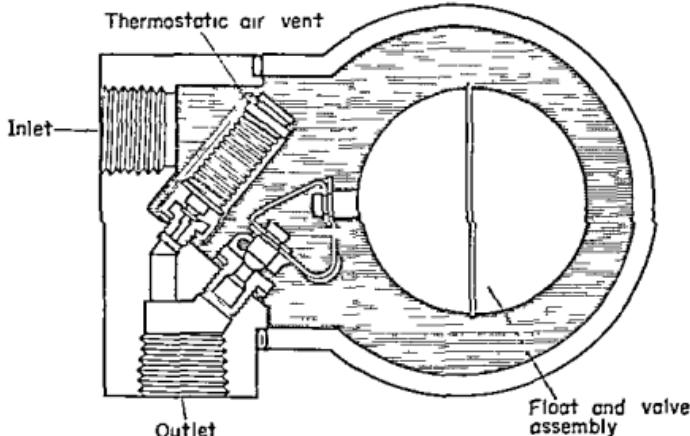


Fig. 22.18 BALL-FLOAT TRAP WITH INTERNAL THERMOSTATIC AIR VENT

(Courtesy Sprax-Sarco Ltd.)

forcing out the condensate. The float then sinks to close the outlet again and prevent unnecessary loss of steam. Because this type of trap will not discharge air, a balanced pressure thermostatic valve is fitted inside it and a by pass should be provided.

The same considerations apply to the sensing of temperature as in dressings sterilisation. Drying the load and admission of air are not relevant.

#### Cooling the Load

A load of bottled fluids cools very slowly because of its large heat capacity and the poor rate of heat transfer through and from the glass walls. If the steriliser is opened before the bottles are below 100°C, some may explode or lose their closures because of their high internal pressure and the shock to the glass of the cool air of the room. Post-sterilisation high vacuum is of little value because it only removes the steam and evaporates residual condensate, while the risk of breakages is considerably increased. Normal procedure has been to allow the steam to vent slowly, during at least 10 minutes, through the discharge channel or its suitably adjusted, full bore by pass. After this the steriliser is left to cool. A large load can take almost a day to cool adequately and, meanwhile, the steriliser is out of use and the contents are receiving a long and, sometimes, injurious exposure to heat.

Recently, Wilkinson, Peacock and Robins (1960) devised a direct method of cooling that gives a tremendous reduction in the time. Immediately after exposure the bottles are drenched in a very fine mist of cold water. No breakages occur if the droplet size is sufficiently small, the optimum for water at 18°C being 50 to 100 microns. Large drops cause severe local cooling, which shocks the glass and leads to fracture. Nozzles of the whirling spray type are used to produce the mist and are arranged to give maximum turbulence. The water should be distilled or deionised to prevent deposits. Compressed air is admitted to the bottom of the chamber to compensate for the pressure drop caused by the condensation of the steam, very little is necessary because the pressure is prevented from falling sharply by the production of steam from the evaporation of the mist.

Table 22.10  
Time for Contents to Fall from 115 to 95°C

Load	Without water cooling	With water cooling
24 × 500 ml bottles	3 hr	10 min
200 × 1 litre bottles	22 hr	17 min

by the hot bottles. The value of the method is indicated in Table 22.10.

The operator has greater safety and less damage is done to the contents, e.g. dextrose solutions are much less discoloured.

Sterilisers incorporating this feature are available.

#### Automatic Control

The successful operation of a steam steriliser requires great care. Disastrous results can follow mistakes such as drawing an inadequate vacuum, using the wrong steam pressure and exposing for an incorrect time. Human errors cannot always be prevented and have been responsible for failures in dressings sterilisation in the past (Nuffield Provincial Hospitals Trust, 1958). Automatic control gives a higher degree of safety and can be provided by most makers of modern sterilisers. Its features include—

(a) A mechanism to prevent the operation of any part of the process until the door is shut and locked.

(b) Most controllers provide several different cycles, e.g. for dressings, bottled fluids, instruments and utensils, and rubber gloves. One of these is selected by pressing the appropriate switch on a control panel and thereafter all stages of that process take place automatically.

(c) Stage 1 Pre vacuum. The pump is switched on and allowed to reduce the chamber pressure to 20 mm Hg abs. It is then stopped by a barometrically compensated switch which ensures the correct residual pressure in spite of variations in barometric height. If the pump always removed the same amount of the chamber pressure the residual would be more than 20 mm when the atmospheric pressure was above 760 mm, and the quantity of sterilisation inhibiting air in pockets and films correspondingly increased.

(d) Stage 2 Heating and Sterilisation. Steam is admitted and when the temperature in the discharge channel or load is at 121°C a time temperature integrator begins to operate. This device automatically relates the exposure time to the temperature. At the maximum of 134°C a 3 min exposure is given, but if the temperature falls the time is automatically increased in accordance with a curve based on thermal death times and a safety factor. The integrator will not work below 121°C, at which it gives an exposure of 15 min. In the bottled fluids cycle, steam is not admitted to the jacket and a special device allows for heating up; in addition, the next two stages are replaced by quick-cooling or slow venting.

(e) *Stage 3 Post vacuum* Steam to the chamber is shut off and the pump restarted. When the post-vacuum has been drawn a barometrically compensated switch changes the process to the last stage.

(f) *Stage 4 Breaking the Vacuum* Filtered air is admitted. When a ceramic filter is used it may be flushed with steam in stage 2.

Many safeguards are provided—

1 A visible or audible alarm operates if—

- (a) The necessary conditions for a switch from one stage to the next are not reached within a reasonable period after the normal time
- (b) If the supply steam pressure is inadequate initially or at any time during the exposure
- (c) If the temperature fails to reach or falls below 121°C

2 It is impossible to interfere manually with the steriliser (except to stop it in an emergency) while it is under automatic control. Provision is made for independent manual operation, a handwheel is used to rotate a camshaft to four positions and operate the four stages in correct sequence.

3 Emergency stopping causes the steam to vent, the controller to reset and the indicator panel to show that the contents are unsterile.

#### DRY-HEAT STERILISATION

The problem is simpler in this case because only the lag and exposure stages are involved. A suitable device is a switch that starts timing when the air temperature is at sterilisation level and turns off the oven after the lag and exposure times have been given. It should not record when the temperature is below the sterilisation value.

### APPLICATIONS

#### I GLASS APPARATUS AND CONTAINERS

It is better to use dry heat for the sterilisation of containers and apparatus made entirely of glass because with moist heat

- (a) Post sterilisation drying is necessary. For this the BPC suggest heating at about 65°C
- (b) Air drainage must be ensured by suitable loading. Ideally, bottles should be inverted
- (c) Extraction from and damage to the glass surfaces is more likely

However, some of the glass equipment used in aseptic technique has rubber parts. If these are of silicone (the only dry heat resistant rubber in common use for tubing) hot-air sterilisation is possible but other rubbers must be autoclaved. The apparatus should be dissembled sufficiently to allow easy entry of steam, e.g. a receiver closed by a bung carrying a bacteria proof filter should not be sterilised with the bung in place. Openings are loosely plugged with muslin-covered non-absorbent cotton wool or protected with a paper cap held on by an elastic band or twine. Wrapping should comply with the principles outlined under dressings, and loading must encourage quick air drainage. If possible, equipment with no large openings (e.g. Fig. 24.23(a)) should be sterilised in an autoclave in which a pre-sterilisation vacuum can be drawn because satisfactory air removal by drainage is unlikely. Glassware must be degreased.

#### II CLOSURES

Normally, dry heat is used for loosely fitting metal caps but metal or thermostable plastic screw caps with rubber liners must be autoclaved. Hermetically-sealed empty containers must not be sterilised by moist heat because the steam cannot penetrate to the inside, the caps should be very loose and the bottle inverted or, preferably, because it avoids the need for drying, the bottle can be separated, paper-capped and sterilised by dry heat. Caps should be arranged in a single layer, to minimise trapping of air, and wrapped in paper or enclosed in a heat-sealed paper bag.

Rubber closures for multi-dose containers are most conveniently autoclaved in the solution with which they have been equilibrated or in water for injection, if equilibration is unnecessary. A jar with a screw cap and suitable liner is necessary to prevent loss of volatile preservatives.

Suitable exposures for glassware and closures are 115°C for 30 min (the *British Pharmacopoeia* method) or 121°C for 15 min but both depend on satisfactory air removal from empty containers, and the time should be extended if this is in doubt.

#### III INJECTION SOLUTIONS AND SUSPENSIONS

There are more than 100 official injections and the majority are sterilised by autoclaving (see Appendix 5). The BPC specifies 115 to 116°C for 30 min, but there can be no serious objection to shorter time at

Table 22 11

	<i>Manually operated, vertical or horizontal</i>	<i>Automatically controlled horizontal</i>
Air removal	Downward displacement	High vacuum
Exposure (preceded by heating-up)	30 min at 115°C	30 min at 115°C or 15 min at 121°C
Cooling	Slow venting of steam followed by very slow cooling	As opposite, or quick cooling by water sprays

a higher temperature (e.g. 15 min at 121°C) if the medicament is sufficiently thermostable (e.g. sodium chloride). Although the medicaments for which autoclaving is recommended have a high degree of thermostability it must not be assumed that it is safe in all cases to increase the temperature, time or both. The evidence that no harm will result must be unquestionable. Generally it is advisable to follow the well-tried B.P. directions. The importance of finding and adding the lag times for large volumes cannot be over-emphasised.

The normal stages of the process in the main types of large steriliser are summarised in Table 22 11 but variations, such as high vacuum air-removal from manually-operated sterilisers are found occasionally.

#### IV SURGICAL DRESSINGS AND FABRICS

This description includes a variety of materials used in surgery and in the ward treatment of wounds and infections. Examples are cotton-wool balls, gauze swabs, ribbon gauze, bandages, operating gowns, caps and masks, towels, trolley cloths and rubber sheeting. Successful sterilisation requires careful selection of containers or packaging material, faultless methods of packing, and correct loading in the steriliser. Particular care is necessary if high vacuum air-removal is not available.

##### A The Container or Packaging Material

###### 1 METAL DRUMS

These are cylindrical or rectangular boxes with well fitting hinged lids. They are made from chromium-plated or nickel plated brass or copper or from stainless steel. To facilitate entry of steam and escape of air they should be perforated, and older types of drum without this feature are unsatisfactory. Until recently the holes were in the sides and could be uncovered or covered, immediately before and after sterilisation respectively, by moving

a sliding band (Fig. 22 19). Now, a rectangular box, designed particularly for rectangular sterilisers, is available and this has perforations in the top and bottom, equivalent to at least 15 per cent of the perforated surface, a fibreglass filter is fitted beneath each set of holes and the lid has a rubber gasket (B.S. 3281 1960 and Thompson and O'Grady, 1959).

The advantages of drums include indestructibility if carefully handled, easy packing and removal of the contents, and good protection of the latter during post-sterilisation storage.

However, they hinder steam penetration and, especially in sterilisers without high vacuum, this may necessitate longer exposures with consequent damage to the outer layers. If the rim, lid or slide becomes dented or distorted inadequate protection is given to the contents. The operator may forget to close the slides after sterilisation. In addition, the ease with which drums can be closed after removal of the items needed for one operation or treatment may give staff the impression that the remainder can be safely used at a later date. Because of the danger of aerial contamination each time the lid is opened this practice is unsafe except, perhaps,

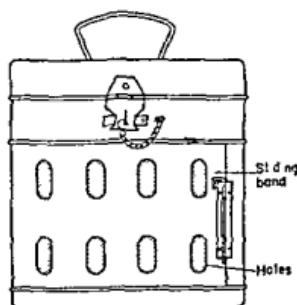


Fig. 22 19 DRESSINGS' DRUM

in operating theatres and other units with filtered air

## 2 CARDBOARD BOXES

These were introduced to overcome some of the disadvantages of metal drums (Nuffield Provincial Hospitals Trust, 1958). They are available in several sizes, have lids that fit snugly over and well down the sides of the box, and the corners are sealed against the entry of dust (Fig 22.20).

Their main advantages are better post sterilisation protection of the contents (the deep lid and protected corners are more efficient anti bacterial barriers than the shallow lid and side of a drum), lightness of weight and ease of stacking. Organisms do not penetrate the undamaged cardboard even in a humid atmosphere. In high vacuum, but not in downward displacement sterilisers, penetration of steam to the centre takes place more quickly than in a drum of comparable size.

Nevertheless, they are not entirely satisfactory. Removal and replacement of the well-fitting lids is not easy. Regular replacement is necessary although with care they last for about 24 autoclave cycles. Unlike drums they cannot be thoroughly cleaned before re-use. If wet steam is inadvertently used they may become soaked, which hinders steam penetration and leaves them stained and weakened. Cardboard is often contaminated with *Clostridia* and although pathogenic strains have not been isolated the possibility of their occurrence cannot be ignored, unless the boxes have been autoclaved by the manu-

facturer before sale they must never be used without sterilisation, not even as a protective post sterilisation container for sterilised wrapped packs (See Alder and Gillespie (1959) and Thompson (1959)).

## 3 WRAPPINGS

### (a) Fabrics

An alternative method of packing surgical dressings is to wrap them in a double thickness of a suitable fabric, such as a good quality muslin, and tie with string or tapes.

The major advantage is almost instantaneous steam penetration. The fabrics can be re-used for some time, and attempts to preserve unused items for a future occasion by remaking the pack are unlikely (see 'Drums').

It is more difficult to keep the contents sterile, dry and free from insect attack than when drums and boxes are used. Alder and Alder (1961) have shown that bacterial contamination occurs less readily when balloon cloth or unbleached calico is used instead of muslin. The wrappings have to be laundered, and preparation of the packs is tedious and requires great care to ensure that the contents are fully enclosed.

### (b) Nylon Film

This is available in the form of tubes of various widths. Suitable lengths can be made into bags by sealing the cut ends with heat (special sealers are necessary) or autoclave-resistant tape. Large tubes can be used to wrap parcels of dressings.

The film is tough, smooth and bacteriologically acceptable in composition and appearance. It is permeable to steam but impermeable to bacteria. It can be used several times and the thickest sheets are not easily damaged by sharp instruments.

Air does not escape quickly and, therefore, heating up is delayed and bags may burst in high vacuum (Edmunds, 1961; Fallon 1961). This problem is reduced if one end of the bag is not heat-sealed but, instead, is folded over twice and taped (Bridgen, 1961). The method is costly.

### (c) Paper

The chance of infection is reduced if each package contains only enough items for one procedure but then the number of sizes becomes considerable because of the very different needs of certain techniques, e.g. a simple ward dressing and a major surgical operation. The use of drums or boxes for a few items is inconvenient and wasteful of space and labour, and fabric packs are not entirely satisfactory because of the risk of post sterilisation

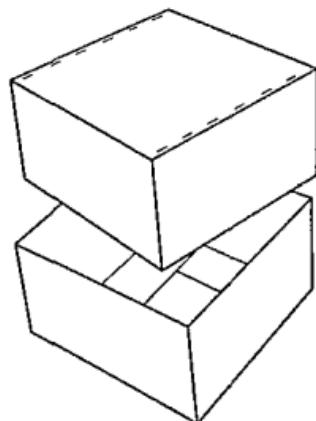


Fig 22.20 CARDBOARD BOX

**infection** A simple way of obtaining the necessary flexibility of size is to use paper bags (Darmady *et al.*, 1960, Welch, 1961)

An acceptable paper must—

- (i) provide a barrier against bacteria An even fibre structure free from pin holes is desirable Glazing, which produces the so-called glassine papers, is helpful,
- (ii) be permeable to steam and air All papers seem to be adequately steam permeable but denser types, e.g. glassines inhibit the escape of air and thus may lead to bursting in high vacuum sterilisers,
- (iii) have sufficient tensile strength to withstand the normal risks of handling,
- (iv) withstand steam sterilisation It must have adequate wet strength and must not undergo serious loss of tensile strength or increase in bacterial permeability

Hunter, Harbord and Ridgett (1961) have discussed these properties and suggested tests for their evaluation

The most suitable material seems to be bleached Kraft, and bags are most often made from this Heavyweight crepes are used for wrapping and glassine bags are also available There is a special paper, water repellent on one side, in which articles can be wrapped before placing in the bags When opened out it lies flat easily and forms a sterile surface from which dressings can be performed The repellent surface prevents contamination from the underlying trolley if the paper becomes wet (Darmady *et al.*, 1960)

Bags are sealed by heat or, less often, by autoclave tape They are disposable, relatively inexpensive and there are no laundry costs However, cracks and large holes are difficult to detect once the contents are inside Although paper is not quite as permeable to steam as a fabric wrap it gives better post sterilisation protection against bacterial contamination (Alder and Alder, 1961)

## B Methods of Packing

The general principles are—

- 1 The pack should be kept as small as possible
- 2 The contents should be arranged loosely
- 3 The spaces between the items and folds should be parallel
- 4 The heavy, impermeable surfaces of rubber sheets should be kept apart with interleaves of pieces of open fabric
- 5 Heavy, tightly woven materials (e.g. certain types of hand towel) and rubber sheets should, if

possible, be sterilised apart from other materials and in small numbers, very loosely arranged

These precautions will ensure satisfactory steam penetration and air removal

### 1 Metal Drums

These should be lined with a double layer of fabric of sufficient size to turn over and cover the dressings at the top This lining filters any air drawn in during cooling or as a result of temperature changes during storage, it may be re used but must be examined for faults each time

Articles are packed parallel to the bottom in drums with side vents, and parallel to the sides in drums with vents at top and bottom, the holes should be left as clear as possible The lid fastener should have a seal that cannot easily be replaced once removed, e.g. a wire sealed with lead

### 2 Cardboard Boxes

To give extra protection the contents should be wrapped in paper, plastic or fabric The spaces between the items should be parallel to the sides of the box

### 3 Fabric Packs

These must not be too large The generally accepted maximum is 12 × 12 × 20 inches Before wrapping in the outer double layer of fabric it is useful to enclose the articles in a trolley towel of cloth or paper to serve as the sterile working surface when the contents are used, it also provides an extra protective barrier during storage

### 4 Nylon Bags

As much air as possible should be pressed out before sealing

### 5 Paper Bags

The articles should be given an inner wrapping of paper, e.g. crepe, as a protection against damage to the bag

## C Loading the Steriliser

Drums with top and bottom vents and cardboard boxes are loaded with lids uppermost Drums with side vents are placed on their sides after opening the slides In all three types the contents will then be on edge Bags and parcels should also be positioned to give the same arrangement As the steam penetrates the heavier air is able to flow freely down and out from between the folds and items In addition pocketing in the bottoms of side vented drums is prevented

Preferably, shelves should be used to separate layers of containers but if this is not possible piling should be staggered and overloading avoided. With a porous load the perforated tray at the bottom of the chamber must not be removed to make more room because air and condensate drainage will be impaired.

Poor packing and loading are said to have very little effect on the efficiency of sterilisation if high vacuum is used. However, as the recommended procedures add to the safety of the method there is a strong case for adopting them generally.

#### D The Sterilisation Cycle

Table 22.12 gives a summary of the usual cycles in the two main types of steriliser.

Table 22.12

	<i>Manually operated</i>	<i>Automatically controlled</i>
Air removal	Double low vacuum with intermediate steam admission or Downward displacement	High vacuum
Exposure	30 or 45 min at 121°C 20 or 30 min at 126°C (see below)	3 min at 134°C
Drying	Warm filtered air through or Low vacuum (Hot jacket assists both)	High vacuum

Some manually operated types have been modified by the addition of a vacuum pump. The shorter of the two exposures at 121° and 126°C is for fabric packs, and the longer for dressing drums. They

include an allowance for heating up because timing is begun when the discharge channel thermometer is within a degree or two of the exposure temperature (see M.R.C. Report 1959).

#### E Removal from the Steriliser

The load is removed as aseptically as possible. Vent slides are closed immediately and warm packs are not placed on a cold surface in case a pool of condensate collects and causes contamination through porous wrappers. Each container is labelled with the date of sterilisation to prevent over long storage but moistened labels are not suitable for permeable surfaces, a blunt, very soft pencil can be used for paper or card.

#### ADVANTAGES OF AUTOCLAVING

- 1 Autoclaving destroys micro-organisms more efficiently than dry heat and, therefore a shorter exposure at a lower temperature is possible.
- 2 It can be used for a large proportion of the official injections.
- 3 In a steriliser supplied with dry saturated steam porous materials can be sterilised without damage.
- 4 Equipment or components of rubber and certain plastics, such as nylon and P.V.C., will withstand the conditions.

#### DISADVANTAGES OF AUTOCLAVING

- 1 It is unsuitable for anhydrous materials such as powders and oils. To protect these from damage by condensate, enclosure in hermetically sealed containers would be necessary, but then the steam would not reach the contents which would receive the inadequate exposure of 30 minutes of dry heat at 115°C.
- 2 It cannot be used for injections and articles such as some plastics, that deteriorate at 115°C. (See also advantages 2 and 3 of 'Dry heat Sterilisation').

#### TESTING THE EFFICIENCY OF STERILISERS

Regular tests on steam sterilisation processes are necessary to confirm that the equipment is working satisfactorily and is being operated correctly. Before these are attempted it is essential to make sure from—

- 1 An engineer—that the steriliser is of satisfactory design, correctly installed and properly instrumented.
- 2 The staff responsible for packaging—that they know the correct methods.

- 3 The operator of the steriliser—that he fully understands the technique. Only then are efficiency tests worthwhile. Two types of test are possible (Kelsey, 1959).

#### I DIRECT TESTS (STERILITY TESTS ON THE PRODUCTS)

It would be useful if the sterility of each load and, therefore, the efficiency of each process, could be proved by simple, rapid and non-destructive tests on every item before it was used. Sterility tests have

none of these advantages, they must be carried out under carefully controlled conditions by highly skilled staff who understand the limitations imposed by the restricted choice of media and incubation conditions, who are aware of the danger of accidental contamination (particularly of dressings) during testing and who are able to interpret their results critically. Probably, only the microbiological control laboratories of pharmaceutical houses provide adequate facilities. Further, it is not possible to test every item and then use it, because sterility testing requires removal of the article or material from its packet or container and, therefore, if the results are to have any meaning, a fairly large number of samples must be sacrificed, this may be inconvenient and wasteful in, say, a small hospital. Finally, the long period necessary for the incubation of the tests delays detection and correction of faults.

Consequently, this is not a very suitable method of testing a steriliser.

## II INDIRECT TESTS

### A Instrumental

The theory underlying this method is that if satisfactory physical conditions can be shown to have existed in the load for an adequate time it is justifiable to assume that the contents will be sterile. Such proof can be obtained only by adequate instrumentation, and ideally this should indicate that—

- 1 The sterilising temperature was maintained throughout the load and for the proper time (in dry heat and steam sterilisers)
- 2 The correct vacua were drawn (particularly in high vacuum steam sterilisers)
- 3 Superheating was not excessive (in steam sterilisers)

With regard to high vacuum and superheating no instruments suitable for autoclaves are in general use yet and reliance must be put on the accuracy of setting and correct functioning of the vacuum switch (vacuum) and the efficiency of the air discharge mechanisms (superheating).

Temperature can be recorded easily, and suitable sensing devices have been mentioned earlier. One suitable method for a steam steriliser is to put the bulb of a second mercury in steel thermometer in the drain and to connect the flexible capillary tube from this, not to a dial as in the indicating thermometer, but to a pen recorder which will make a continuous trace of the temperature on a moving chart. The chart speed should be fast enough to give a large trace on which times can be measured accurately.

The thermometer method is not entirely satisfactory because the temperature is not sensed from inside the load. The alternative is to put thermocouples or thermistors inside dummy packs made up to simulate the least penetrable containers in the chamber (e.g. a large drum containing heavy fabrics) and placed in the coolest parts (e.g. at the bottom near to the drain of a downward displacement steriliser). The outputs from these can be used to operate several recorders. This equipment is more expensive but gives very quick responses to temperature changes.

If the charts are regularly checked after each cycle faults will be detected immediately and there will be no danger of issuing unsterile products.

Compared with other means of testing, this method has been criticised on three main grounds, cost, complexity and its failure to indicate unsatisfactory conditions of humidity (e.g. superheating). Nevertheless, the accuracy of the information it can provide on heating-up rates, temperature distributions and exposure times makes it invaluable.

Instrumentation, like automation may seem to have lessened human responsibility for sterilising processes. In fact, this has merely been transferred to the checking of the complex control equipment and the supervision of its maintenance. Regular inspection is necessary to detect fatigued gauges, kinked capillaries, distorted pens, blocked strainers, dirty contacts, clogged air filters and inaccurate vacuum switches, all of which can lead to serious errors.

### B. Cultural

Preparations containing bacterial spores are put into dummy packs in the load and tested for sterility after exposure. The method has the advantage of clearly demonstrating that the process is fulfilling its purpose, the destruction of micro-organisms. Two main problems complicate its application.

#### 1 SELECTION OF SPORES

Selected spores must not be less resistant than those of the most resistant pathogens. For many years sterilisers were tested with the mesophile *Bacillus subtilis* but this was an unwise choice because the spores of most strains can be killed in a few minutes in boiling water. The pathogens are too dangerous for routine use, and the remaining alternatives are soil samples or thermophilic bacteria.

#### (a) Soil Samples

In some soils the organisms are of satisfactory resistance but in others they are too heat sensitive,

consequently, tests for suitability are necessary. The reading of the sterility tests is complicated by the turbidity created by the soil particles, and subculturing may be unsuccessful if the organisms need growth factors from the soil because the amounts transferred in the subcultures may not be sufficient. One solution is to examine the original tubes microscopically, and another is to incorporate the sample in a plate of solid culture medium on which surface colonies, at least, will be distinguishable. The soil organisms should not be wetter than the organisms in, or on, the article being sterilised. This is especially important in the testing of dry-heat sterilisation, to prevent any moist heat contribution to the death rate. Air drying the soil may be sufficient but better control of the moisture content is obtained by using a vacuum desiccator containing calcium chloride. The particle size of the soil can be standardised by sieving.

#### (b) Thermophilic Bacteria

The use of a specific thermophile has several advantages: cultivation and spore production can be accurately controlled to minimise variation in resistance, there is no non-specific turbidity during sterility testing and it is simpler to provide a definite number of spores for inoculating a test paper. It is an advantage for a central reference laboratory or a commercial source to undertake the supply of suitable preparations of standard resistance and in this country a test paper for steam sterilisers is obtainable from the Oxo division of Oxo Ltd. The spores of *Bacillus stearothermophilus* are used and are claimed to survive 5, but not 12 minutes of moist heat at 121°C. This provides a margin of safety because the spores of the most resistant pathogens are destroyed by 4 minutes at this temperature.

As the time necessary to sterilise an article depends on the number of organisms initially present, a reasonably heavy challenge should be provided, the spore papers contain approximately  $10^5$ . In chemical methods of sterilisation the surface on which the spores are exposed influences the rate of kill, but this is not important for heat methods.

There is no commercial test preparation for dry-heat sterilisation but Darmady, Hughes and Jones (1958) recommended the use of a non toxicogenic strain of *Clostridium tetani*.

#### 2 STERILITY TESTING

Commercial papers consist of thick filter paper strips impregnated with the spores and usually they are sterilised inside glassine envelopes. Although the

subsequent tests for sterility are straightforward the usual precautions must be taken as the following examples show—

- The medium must satisfy the nutritional requirements of the organisms. The importance of the composition of the medium in the recovery of *Bacillus stearothermophilus* has been shown by Cook and Brown (1960, 1962).
- The incubation conditions must be optimal. *Bacillus stearothermophilus* is a thermophile and requires a temperature of 55 to 60°C. Seven days is the recommended time but the work of Cook and Brown suggests that this could be reduced, which would be an advantage because one fault of cultural tests is the long wait for the results.
- Several strips should be placed in each site in the steriliser to prevent an incorrect conclusion based on a paper of unusual resistance.
- The removal of the strips from their envelopes to the test medium may result in accidental contamination. The technique should be checked from time to time (see 'Sterility Testing of Dressings') (See also Heden and Markkula, 1962.)

#### C. Chemical

Chemical indicators fall into two main classes

##### 1 Types that cannot Indicate a Satisfactory Exposure

The earliest, called witness tubes, were sealed glass tubes containing a chemical that melted at sterilisation temperature, e.g. acetanilide for 115°C and benzoic acid for 121°C. Sometimes, to facilitate recognition of the change, a dye such as methylene blue that gave a different colour when dissolved in the melt, was included. They passed out of use in this form because they gave no indication of the time of exposure.

The following two types have the advantage over witness tubes of not being affected by dry heat.

*Klintex Papers* (Robert Whitelaw Ltd., Newcastle, 2) These are paper strips with a coloured coating that disappears in the presence of steam, leaving the word 'Autoclaved' in black on a pale background. Brown and Ridout (1960) showed that 2½ min over boiling water would cause the change.

*Test Tablets* (British Chemotheutic Products Ltd, Bradford). These contain 75 per cent lactose, 24 per cent starch and 1 per cent magnesium trisilicate. They are hard and white at first but autoclaving makes them brown and gelatinous, without loss of shape. The change takes place after 24 min at 115°C.

They should be examined soon after removal from the steriliser because they gradually harden again until, after 7 days, the only remaining signs of the heat treatment are a slight discolouration and a wrinkled surface.

Since neither of these devices can indicate that an article has received a sterilising exposure they should only be used to demonstrate that a container has not been left out of the steriliser by mistake. They are put in the tops of packs and drums where they are seen as soon as the closure is opened.

## 2 Types that Indicate a Satisfactory Exposure

Kelsey (1958, 1959) has shown that when the thermal death times in moist heat of heat-resistant sporing pathogens and saprophytes are plotted on a graph of temperature against the logarithm of the death time, the points for any particular organism lie almost on a straight line, the slope of which corresponds approximately to a tenfold decrease in time for a  $10^{\circ}\text{C}$  rise in temperature (compare Rahn's results, p. 324). Only exposures on or above this line will kill the organism. He suggested that a suitable performance for a test object would be represented by a line parallel to but above the line adjoining the thermal death times of the most resistant pathogen. The distance between them should be sufficient to give an adequate safety margin but smaller than the distance between the thermal death time line and a line joining normal sterilising exposures. If the object performance line corresponded with the sterilisation exposure line it would be rare to get a satisfactory test result in practice. Kelsey located his object line to pass through 1 min at  $128^{\circ}\text{C}$ , 5 min at  $121^{\circ}\text{C}$  and 20 min at  $115^{\circ}\text{C}$  (Fig. 22.21).

Darmady, Hughes and Jones (1958) made a similar investigation for dry heat sterilisation and also recommended characteristics for a suitable indicator. In this case the slope for the most resistant pathogen corresponded to only a fourfold decrease

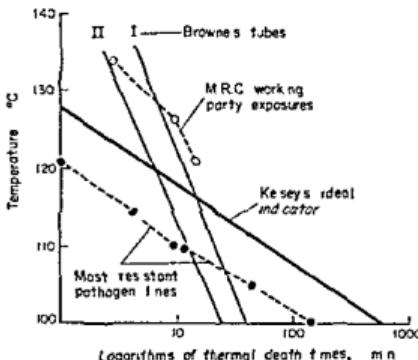


Fig. 22.21 (Adapted from Kelsey 1958 1959)

in time for a  $10^{\circ}\text{C}$  rise in temperature, a difference that would be expected because of the different mechanism of destruction.

*Browne's Tubes* (Albert Browne Ltd., Leicester) These are widely used in this country. A sealed glass tube contains a red fluid that changes through amber to green on heating. They are reported to depend on the hydrolysis of an ester. Types are available for use in ordinary steam sterilisers, high vacuum sterilisers, hot air ovens and infra-red conveyor ovens (I to IV respectively). Some of their characteristics (taken from the manufacturer's literature) are shown in Table 22.13.

The slopes of the characteristic curves of types I and II do not fit Kelsey's object performance line very closely, the safety margin being too wide at high and too narrow at low temperatures (Fig. 22.21). However, type I is reasonably satisfactory at  $115^{\circ}\text{C}$  and type II at  $121^{\circ}\text{C}$  and above. The dry-heat types also have a wide safety margin at high temperatures. Type III is adequate at  $160^{\circ}\text{C}$ .

Table 22.13  
Steam sterilisation types      Dry-heat types

Temperature (°C)	Time for full green		Temperature (°C)	Time for full green	
	I (min)	II (min)		III (min)	IV (min)
130	6.5	3.5	190	8	6
125	10.0	5.5	180	16	12
120	16.0	9.0	170	31	24
115	25.0	15.0	160	60	45
100	100.0	66.0	150	115	85

but is unsuitable at 150°C. Darmady has recommended exposures of 7.5 min at 180°C and 1.5 min at 190°C for infra-red sterilisation and, therefore, for this process, type IV has more suitable characteristics than type III.

Brown and Ridout (1960) investigated this type of indicator and made several recommendations—

- 1 The tubes must be stored below 20°C before use. At higher temperatures the hydrolysis begins and, therefore, the time for the colour change in the steriliser is reduced.
- 2 They should be examined as soon as possible after sterilisation because the colour alters on subsequent storage.
- 3 Colour standards should be used to assist the interpretation of 'full green'. This is very important because the time to produce the first sign of green is much less than the time to give the full colour.

They are unreliable when a considerable heating-up time is necessary, e.g. for a load of bottled fluids, unless they are suspended at the centre of dummy containers.

*Hour glass Devices* Like the earliest witness tubes, these make use of the melting point of a solid but with modifications to introduce a time factor. The first (Fig. 22.22a) was designed by Brewer and McLoughlin, the amount of solid and the capillary diameter were chosen so that the exposure time was required for all the material to flow into the lower chamber.

Royce (1959) decided that the side arm, through which the displaced air escapes, was too fragile, and he invented a tube with a pinch constriction in the middle (Fig. 22.22b). When this is set at an angle in a stand the melted solid runs down one side of the constriction while the air escapes up the other. He used acetanilide for 115°C, succinic anhydride for 121°C and para-acetotoluolide for 150°C (the last for dry-heat sterilisation).

These types cannot be defeated by sub-lethal heating for a longer time but are vulnerable to a

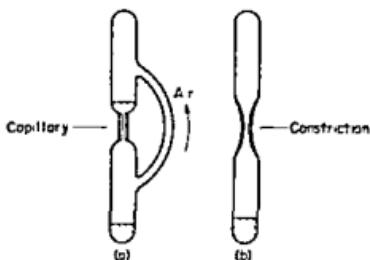


Fig. 22.22 HOUR-GLASS DEVICES

(a) Brewer and McLoughlin (b) Royce

short-time heating at more than about 5°C above the operating temperature. For example, at 115°C the acetanilide takes 30 min to pass through, but at 121°C only 5 min is required. They can be re-used but, because correct positioning is necessary, it is unsafe to put them inside opaque containers.

Neither hour-glasses nor Browne's tubes will indicate over-exposure or the humidity conditions. Their chief advantage over cultural methods is that they can be read immediately.

The reliability and immediate availability of the information given by accurate recording instruments makes them the method of choice, and the development of more convenient equipment for sensing from the load, e.g. the West sterilisation integrator (Bowie, 1961), together with devices for the measurement of superheating (Shotton, 1961) will strengthen their position.

Cultural indicators are useful to confirm that conditions are satisfactory, particularly in parts of the chamber or load that instruments have shown to be cooler than the rest.

It is probable that as methods of automation, sensing and recording improve, chemical indicators, because of their relative lack of precision, will be used solely to confirm that articles have been in the steriliser.

## OTHER METHODS OF STERILISATION BY MOIST HEAT

### 1. STERILISATION OF VACCINES

One type of vaccine is a suspension of dead bacteria. The method used to kill the organisms depends on the stability of their antigens, because little or no immunity is produced if these are damaged. Sometimes treatment with liquid bactericides at room or low temperatures is preferred and examples will be

found in the *British Pharmacopoeia* but, often, better immunological responses are obtained from heat-killed suspensions.

The moist-heat methods used for injections and dressings are too drastic, but since most antigens are stable at about 60°C suitable exposures can be derived from the thermal death time of the organism at or near this temperature by adding a safety factor

Examples are 15 min at 55°C (plague), 1 hr at 56°C (cholera) and 30 min at 60°C (typhoid) autogenous vaccines of *Staphylococcus aureus* are still used occasionally and while most strains are killed by 1 hr at 62°C, some require several minutes at 70°C

These exposures will not kill all other bacteria, particularly sporing species, and the strictest aseptic technique must be maintained before and after sterilisation to exclude all contaminants. In addition, the vaccine is tested for sterility to confirm the success of the process and precautions.

Sterilisation is carried out in a water bath thermostatically controlled to better than  $\pm 1^\circ\text{C}$  of the set temperature. The container must be immersed entirely because organisms on surfaces above the water level may not be killed. An ampoule or tube, sealed by fusion, is most suitable since unsterile water from the bath might leak into a vaccine bottle. The container should be weighted down to prevent floating.

(For additional information on antigens and vaccines see Cooper and Gunn 1957)

## 2 PASTEURISATION

Pasteurisation processes are used to make milk safe and improve its keeping properties. The original method was developed by Pasteur to prevent the souring of wine. He discovered that the responsible micro-organisms could be killed by heating for a few minutes at 50 to 60°C.

When the urgency of preventing the spread of bovine tuberculosis to humans was appreciated a method was required that would kill the tubercle bacillus without significantly affecting the taste, lowering the nutritional value, reducing the cream layer or precipitating the proteins of the milk. To preserve these properties a low temperature process is essential and Pasteur's technique provided a valuable starting point for the development of the main methods used today.

### (a) The Holder Method

The milk is heated at 145°F (62.8°C) held there for 30 min and quickly cooled. This is carried out in jacketed stainless steel tanks containing agitators to ensure the correct exposure throughout the milk, and to prevent skin formation. Clean, dry steam is admitted to the space above the liquid to collapse the foam. Organisms in the skin or foam may escape sterilisation. A special application of this method is the pasteurisation of human milk donated to milk banks of maternity units of major hospitals, afterwards the milk can be kept for about 6 months at  $-20^\circ\text{C}$ .

The milk proteins tend to protect micro-organisms but, in spite of this, *Mycobacterium tuberculosis* is killed in 20 min at 145°F, and the extra 10 minutes provides a very safe exposure which also destroys the other pathogens found in milk and the lactic acid producing organisms responsible for souring.

### (b) The High temperature, Short time (H.T.S.T. or 'Flash') Method

The milk is rapidly raised to 161°F (71.6°C), held at this temperature for at least 15 sec and quickly cooled. Two major types of equipment are used. The first has a series of thin, vertical, rectangular, channelled, stainless steel plates and the milk passes through the alternate spaces while the heating water flows in the opposite direction through the others. In the second, the milk flows through narrow horizontal pipes inside larger ones through which the water passes in the opposite direction. Pipes are more difficult to clean and their withdrawal for this purpose requires considerable space.

H.T.S.T. pasteurisers are used by most firms because they save time, are continuous and need less floor space than holding tanks. Automatic control is essential since small human errors can result in an unsafe or heat damaged product.

Although these methods reduce the number of organisms by 97 to 99 per cent they do not destroy sporing or thermophilic bacteria. Therefore, they are unsuitable for sterilising fluids for pharmaceutical purposes. However, if the pasteurisation exposure is replaced by a sterilising exposure, the H.T.S.T. method offers the advantages of speed, continuity and reduced thermal damage when large volumes have to be treated. In the fermentation industry, such a technique is gradually replacing batch sterilisation for the tens of thousands of gallons of culture medium needed for each fermenter (Whitmarsh 1958).

The efficiency of pasteurisation is confirmed indirectly. A slightly more severe heat treatment is required to inactivate the enzyme phosphatase in milk than to destroy *Mycobacterium tuberculosis*. The pasteurised milk is incubated with disodium phenyl phosphate and, if active phosphatase remains, indicating a failure of the process, phenol is liberated and can be detected colorimetrically.

(For additional information on pasteurisation, see Capstick, 1954.)

## 3 TYNDALLISATION

This method was included in the 1932 *British Pharmacopoeia* for the sterilisation of medicaments unstable at 115°C but able to withstand low temperature

heating It was a modification of a technique developed by the bacteriologist Tyndall for killing bacteria in vegetable infusions

The official directions were to pack and seal the solution in its final container and maintain the whole at 80°C for 1 hr on each of three successive days In theory, the first heating destroys the vegetative cells but not the bacterial spores During the interval between the first and second heatings the spores germinate, forming vegetative cells that are killed by the second heating The third heating is a precautionary measure to destroy cells from any spores that do not germinate until the second interval

Within a year or two, Davis (1934, 1935, 1940) had proved that the process was unsuitable for injections because these, unlike the nutritive solutions used by Tyndall, do not provide satisfactory conditions for spore germination The reverse is often the case, not only is an injection solution usually non-nutritive, it may also have an unfavourable pH, an inhibitory medicament or contain a bactericide Storage, during the intervals, at a temperature that would encourage germination was not specified and the conditions were unsuitable for the development of anaerobic spores By carrying out sterility tests after the third heating Davis showed that unless the solution was antibacterial the method could not be relied upon to kill spores

In an attempt to rescue the process, the first addendum to the 1932 B P directed the use of previously sterilised containers and the practice of good aseptic technique, the aim was to prevent contamination by spores However, since these precautions considerably complicated the method its value was greatly reduced Even in its original form it was not very convenient, with the equipment available at the time 80°C was a difficult temperature to maintain accurately and the total sterilisation period was long It was not certain that it was always fulfilling its main function of reducing thermal damage to medicaments because a long exposure to a low temperature may be more harmful than a short time at a high temperature, when the three heating-up and cooling down periods are added to the three hours at 80°C the total heat exposure is considerable Also, the idea of encouraging spore germination in a parenteral solution is objectionable because of the possibility of pyrogen production Following the development of 'Heating with a Bactericide' it was deleted from the *British Pharmacopœia* (Fourth Addendum, 1941)

Nevertheless, it remains a useful process for culture media containing heat sensitive ingredients such as gelatin and sugars Bacteriologists use

Tyndall's original temperature of approximately 100°C and give exposures of from 20 min (for 100 ml) to 45 min, usually in a steamer During the intervals the media should be kept at a warm temperature but, even then, the spores of thermophiles, anaerobes and nutritionally exacting bacteria may fail to germinate

#### 4 HEATING WITH A BACTERICIDE

The failure of Tyndallisation renewed the need for a low-temperature method capable of killing spores without harming heat-sensitive medicaments

It had long been known that bactericides were more effective at high than low temperatures A method of sterilisation based on this fact was proposed by Coulthard (1934) He showed that heating at 80°C for 1 hr in the presence of brilliant green, 1 in 3 000, Solution of Formaldehyde B P, 1 in 500, or mercuric chloride, 1 in 10,000 would sterilise a solution heavily contaminated with spore-bearing organisms, but these substances were unsuitable for general use because they reacted with many medicaments or were too toxic Later (1939) he investigated the use of chlorocresol and phenylmercuric nitrate and obtained results strongly suggesting that the inclusion of a small quantity of either of these compounds would enable solutions heavily contaminated with bacterial spores to be sterilised by heating for  $\frac{1}{2}$  hr at 100°C He drew attention to the low toxicity of these substances

Berry, Jensen and Siller (1938), using a highly resistant strain of *Bacillus subtilis*, found that the addition of 0.25 per cent chlorocresol or 0.001 per cent phenyl mercuric nitrate, followed by immersion in boiling water for 30 min, produced sterility They also listed the properties that substances used as adjuncts in sterilisation should exhibit—

- 1 Freedom from toxicity
- 2 Compatibility with medicaments
- 3 Stability and activity at varying pH values
- 4 Stability during heating and storage

Wien (1939) has shown, by feeding and injection tests on animals, that chlorocresol and phenylmercuric nitrate are non-toxic in the concentrations suggested for use in injections and his results have been confirmed at higher levels by Swift (1950) The other requirements, apart from the limitations discussed earlier in connexion with compatibility, are also satisfied by these compounds

The concentrations finally chosen were 0.2 per cent chlorocresol and 0.002 per cent phenylmercuric nitrate The appropriate amount is included in the solution which, after clarification, is transferred to

the final containers, these, after sealing, are heated at 98° to 100°C for 30 min. This method was named Heating with a Bactericide. It replaced Tyndallisation in the B.P. and is still official today. Apart from one special case (see *Dimenhydrinate Injection*) only two bactericides are permitted by the *British Pharmacopœia* (The *International Pharmacopœia*, which also recognises the method, permits phenylmercuric borate in addition, because this salt is more soluble than the nitrate). Confusion sometimes arises because four substances are recommended as bactericides for the preservation of multiple-dose injections. It will be noticed that to convert the relatively slow bactericidal effect of a preservative into the rapid *sporicidal* action required in a sterilisation technique it has been necessary to increase the concentrations of the two permitted bactericides and to raise the temperature.

*Dimenhydrinate Injection* is a solution of the medicament in 50 per cent propylene glycol. It contains 5 per cent of benzyl alcohol as an anaesthetic because intramuscular injection of propylene glycol is painful; this concentration is more than five times that normally added when benzyl alcohol is used as a preservative in a multiple-dose container. Consequently, it is possible to sterilise the injection by heating at 98–100°C for half an hour.

*Procaine and Adrenaline Injection B.P.* contains only 0.1 per cent of chlorocresol. This is because the summed antibacterial activities of the acid pH (4.3), the antioxidant and the medicaments give with the reduced concentration of chlorocresol a solution with adequate bactericidal activity at 98 to 100°C.

In view of earlier reference to the ability of certain saprophytic spore-producers to survive dry heat sterilisation and autoclaving, it is not surprising that Davies and Davidson (1947) and Davison (1951) found non-pathogenic spores resistant to Heating with a Bactericide. However, because the method is known to destroy pathogens, and no contamination was found in tests on routinely prepared products from hospitals, their discovery has not been considered sufficient reason for altering the process. Nevertheless, it underlines the need for scrupulous cleanliness in the preparation of injection solutions to minimise contamination prior to sterilisation.

#### APPARATUS

##### (a) A Covered Beaker

Heating may be carried out on a small scale by immersion in boiling water contained in a rather large beaker, a clock glass being placed over the top to maintain an atmosphere of steam around any part of the container that may become exposed by

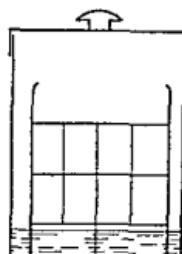


Fig. 22.23 STEAMER

floating to the surface, as often happens with ampoules.

It is safer to restrict this method to ampoules because there is a slight risk that, as a result of an ill fitting outer cap and a damaged bottle neck, water from the beaker may contaminate the injection when a multi-dose container is heated in this way.

##### (b) A Steamer

This is a cylindrical vessel, with a loose fitting lid, containing a basket similar to the one in a small autoclave (Fig. 22.23). About 2 in. of water is put inside and the products to be heated are placed in the basket. An inexpensive alternative is a porringer with holes drilled in the inner vessel (Parkinson, 1961). The apparatus is heated over a ring burner to keep the water boiling vigorously. The lid must be kept closed during the steaming process because if it is tilted or removed the contents may not reach the required temperature. The steamer is suitable for injections in either single or multi dose containers.

##### (c) Electric Boiling water Sterilisers

Although a simple steamer is perfectly satisfactory, more elaborate equipment is useful for larger batches of injections. Modern electric boiling water sterilisers (B.S. 2904 1957) heat quickly, use the minimum current to keep the water boiling and have a safety cut-out that operates if the water level falls dangerously. A valuable feature is the mechanism that automatically closes the lid as the tray is lowered inside (Fig. 22.24).

The most serious fault in carrying out this process is to leave the apparatus completely or partially uncovered. Then the atmosphere above the water becomes a mixture of steam and air and the temperature falls startlingly, e.g. to only 60°C at less than  $\frac{1}{2}$  in. above the surface of the boiling water. As a result, floating ampoules in a beaker or items in the basket of a steamer will not be sterilised.

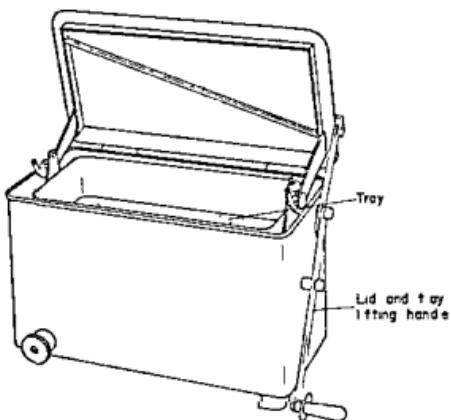


Fig 22.24 ELECTRIC BOILING WATER STERILISER

(Courtesy General Electric Co Ltd)

It is equally important to keep the water boiling

#### Applications

1 It is the official method for solutions of those medicaments that are too thermolabile to withstand sterilisation in an autoclave but are sufficiently stable to be heated at 98° to 100°C. (For examples, see Appendix 4) The whole of the solution must be maintained at this temperature for 30 minutes and, therefore, when the volume is greater than about 30 ml the time must be extended to allow for the lag in heat transference to the centre of the liquid. This lag can be found in a previous experiment. It is negligible for volumes smaller than 30 ml provided the injection and the water in the sterilising apparatus are heated up together.

2 The third method of sterilising containers in the *British Pharmacopoeia* involves immersion in 0.2 per cent chlorocresol, boiling under a reflux condenser for 30 min, and subsequent washing with Water for Injection under aseptic conditions. This is not intended as an alternative to dry heat or autoclaving for glassware but is included to provide a simple process by which certain types of plastic container and equipment can be sterilised without deformation. The risk of contamination during aseptic washing and the fact that the container is left wet are serious disadvantages.

#### ADVANTAGES

- 1 The comparatively low temperature
- 2 The apparatus is inexpensive and simple

#### DISADVANTAGES

1 It cannot be used for solutions or suspensions of medicaments unstable at 98° to 100°C.

2 A substance is added. Ideally, the patient should receive only the medicament prescribed. The official attitude is that injections, in common with all medicines, should, if possible, be prepared by methods that do not involve the addition of other substances. Hence, Heating with a Bactericide must not be regarded as a permissible alternative method of sterilisation for those substances directed to be sterilised by autoclaving.

3 Each bactericide has a number of incompatibilities. These are important in three of the total of twelve B.P., B.P.C. and B.N.F. injections that are sterilised by this method—

Mersalyl and Papaveretum are incompatible with chloroform.

Ascorbic acid is incompatible with phenylmercuric nitrate.

Since, so far as official injections are concerned, this method may be used only when directed, the possibility of incompatibility is chiefly of importance to the pharmacist formulating an injection of a new medicament that is unstable at 115° but stable at 100°C.

4 It is unsuitable for oily solutions and suspensions. The contents of the containers would be subjected to inefficient dry heat at 100°C. Also, the efficiency of the bactericides is lowered in oil (see P. 260).

5 It cannot be used for intravenous injections of dose greater than 15 ml or for intrathecal, peridural and intracisternal injections of any size. (The reasons were given in the discussion of bactericides as preservatives in multiple-dose injections.)

#### REFERENCES

- 1 ALDER, V G and ALDER, F L (1961) Preserving the sterility of surgical dressings wrapped in paper and other materials. *J clin Path*, 14, 76-79
- 2 ALDER, V G and GILLESPIE, W A (1959) Card board boxes in operating theatres. *Lancet*, i, 1209
- 3 BARSON, T E, PEACOCK, F, ROBINS, E L and WILKINSON, G R (1958) The factors influencing sterilisation by low pressure steam. Part I Design and instrumentation. *J Pharm Pharmacol*, 10, 47-55T
- 4 BATESON, F R C, BOYD, J A and GUNN, C (1958) The sterilisation of talcum powder. *Pharm J*, 126, 334

- 5 BERRY, H., JENSEN, E and SILLER, F K (1938) The sterilisation of thermolabile substances in the presence of bactericides *Quart J Pharm* 11, 729-735
- 6 BIGELOW, W D and ESTY, J R (1920) The thermal death point in relation to time of thermophilic organisms *J Infect Dis* 27, 602-617
- 7 BOWIE, J H (1955) Modern apparatus for sterilisation *Pharm J*, 174, 473-476, 489-492
- 7a BOWIE, J H (1959) Operation and use of sterilising equipment *The operation of sterilising autoclaves* The Pharmaceutical Press, London 28-41
- 8 BOWIE, J H (1961) The control of heat sterilisers *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London 109-142
- 9 BRIDGEN, R J (1961) Steam penetration into containers for surgical dressings *Lancet* 2, 207-208
- 10 BRITISH STANDARD 2648 1955 Performance requirements for electrically heated laboratory drying ovens British Standards Institution, London
- 11 BRITISH STANDARD 2904 1957 Hospital sterilisers, boiling water type British Standards Institution London
- 12 BRITISH STANDARD 3219 1960 Horizontal cylindrical hospital sterilisers British Standards Institution, London
- 13 BRITISH STANDARD 3220 1960 Horizontal rectangular hospital sterilisers British Standards Institution, London
- 14 BRITISH STANDARD 3281 1960 Rectangular metal boxes for use in high vacuum steam sterilisers British Standards Institution, London
- 15 BRITISH STANDARD 3421 1961 Performance of electrically heated sterilising ovens British Standards Institution, London
- 16 BROWN, W R L and RIDOUT, C W (1960) An investigation of some sterilisation indicators *Pharm J* 184, 5-8
- 17 CAPSTICK, E (1954) Chemical engineering methods in the food industry Sterilisation and pasteurisation *Chem & Ind Oct 9th* 1242-1248
- 18 COOK, A M and BROWN, M R W (1960) Preliminary studies of the heat resistance of spores on paper carriers *J Pharm Pharmacol* 12, 116-118T
- 19 COOK, A M and BROWN, M R W (1962) Apparatus for testing the resistance to wet heat of bacterial spores in paper carriers *Ibid* 14, 61-62
- 20 COOPER, J W and GUNN, C (1957) *Tutorial Pharmacy* 5th Ed Pitman Medical, London
- 21 COULTHARD, C E (1934) The effect of germicides at 80°C *Pharm J* 133, 447-448
- 22 COULTHARD, C E (1935) Sterilisation by dry heat at 150°C with special reference to oils *Quart J Pharm* 8, 90-93
- 23 COULTHARD, C E (1939) The destruction of bacterial spores Low temperature sterilisation *Pharm J* 142, 79-81
- 24 CRAIK, D J (1958) The flow properties of starch powders and mixtures *J Pharm Pharmacol* 10, 73-79
- 25 CRAIK, D J and MILLER, B F (1958) The flow properties of powders under humid conditions *J Pharm Pharmacol* 10, 136-142T
- 26 DARMADY, E M and BROCK, R B (1954) Temperature levels in hot air ovens *J clin Path* 7, 290
- 27 DARMADY, E M, HUGHES, K E A, and JONES, J D (1958) Thermal death time of spores in dry heat *Lancet* 2, 766-769
- 28 DARMADY, E M, HUGHES, K E A, TUKE, W and VERDON, P (1960) Central sterile supply *The Hospital* 56, 824-838
- 29 DAVIES, G E and DAVISON, J E (1947) The use of antiseptics in the sterilisation of solutions for injection I The efficiency of chlorocresol *Quart J Pharm* 20, 212-218
- 30 DAVIS, H (1934) The preparation of sterile solutions *Quart J Pharm* 7, 379-388
- 31 DAVIS, H (1935) The preparation of sterile solutions II *Quart J Pharm* 8, 361-369
- 32 DAVIS, H (1940) A quantitative bacteriological investigation of the Tyndallisation process *Quart J Pharm* 13, 14-31
- 33 DAVISON, J E (1951) The use of antiseptics in the sterilisation of solutions for injection II The efficiency of phenylmercuric nitrate *J Pharm Pharmacol* 3, 734-740
- 34 EDMUND, P N (1961) Nylon wrapping for steam sterilised dressings *Lancet* 2, 208
- 35 EXTRA PHARMACOPEIA (1955) Vol II 23rd. Ed The Pharmaceutical Press, London 917
- 36 FALLON, R J (1961) Steam penetration into containers for surgical dressings *Lancet* 2, 41-43
- 37 GERSHENFELD, L., MCCLENAHAN, W S and YARLETT, M A (1954) Petrolatum gauze II Its preparation and sterilisation *Drug Standards* 22, 210-215
- 38 GRAINGER, H S and SMITH, M D (1958) Temperature variation in ovens used for sterilising pharmaceutical products *Publ Pharm* 15, 39-43
- 39 GUNN, D C (1961) Estimating steam quality (Abstracted in *Pharm J* 188, 153 154)

- 40 HARRIS, H. F and ALLISON, V. D (1961) Steam sterilisation *Lancet* 2, 603-604
- 41 HEDEV, C. G and MARKKULA, H. (1962) A simple bacteriological test for sterilisation equipment *Proceedings of the 7th International Congress for Microbiological Standardisation* Livingstone, Edinburgh, 215-219
- 42 HENRY, F. S. H. (1959) Physical aspects of sterilising cotton articles by steam. *J appl Bact* 22, 159-173
- 43 HOWIE, J. W. (1961) The surgeon's autoclave *J clin Path* 14, 49-54
- 44 HOWIE, J. W. and TIMBURY, M. (1956) Laboratory tests of operating theatre sterilisers *Lancet* 2, 669-673
- 45 HUNTER, C. L. F., HARBORD, P. E and RIDDETT, D. J. (1961) Packaging papers as bacterial barriers *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London, 166-172
- 46 JORDAN, R. C., JACOBS, S. E. and DAVIS, H. E. F. (1947) Studies in the dynamics of disinfection. The effect of lethal temperatures on standard cultures of *Bact. coli*. *J Hyg, Camb* 45, 136-143, 342-353
- 47 KELSEY, J. C. (1958) The testing of sterilisers *Lancet* 1, 306-309
- 48 KELSEY, J. C. (1959) Methods of testing the bactericidal efficiency of steam sterilisers *Symposium on the operation of sterilising autoclaves* The Pharmaceutical Press, London, 22-28
- 49 KNOX, R., PENIKETT, E. J. K. and DUNCAN, M. E. (1960) The avoidance of excessive superheating during steam sterilisation of dressings *J appl Bact* 23, 21-27
- 50 LEWITH, S. (1890) Arch. exp. Path. Pharmak. 26, 341
- 51 LYLE, O. (1947) *The efficient use of steam* Her Majesty's Stationery Office, London
- 52 M.R.C. REPORT (1959) Report by the Medical Research Council Working Party on pressure-steam sterilisers *Lancet* 1, 425-435
- 53 M.R.C. REPORT (1960) Report by the Medical Research Council Working Party on pressure-steam sterilisers (Second communication) *Lancet* 2, 1243-1244
- 54 NORTHCROFT, L. G. (1952) *Steam trapping and air venting* 3rd Ed. Hutchinson.
- 55 NUFFIELD PROVINCIAL HOSPITALS TRUST (1958) *Present sterilising practice in six hospitals* Nuffield Provincial Hospitals Trust, London. 75p
- 56 O'BRIEN, R. A. and PARISH, H. J. (1935) A note on the sterilisation of oils *Quart J Pharm* 8, 94-95
- 57 PARKINSON, J. (1961) Letter to *Pharmacy Digest* 25, 274
- 58 PENIKETT, E. J. K., ROWE, T. W and ROBSON, E. (1958) Vacuum drying of steam sterilised dressings *J appl Bact* 21, 282-290
- 59 PERKINS, J. J. (1956) *Principles and methods of sterilisation* 1st. Ed. Thomas, Springfield, Illinois
- 60 RAHN, O. (1945) Physical methods of sterilisation of micro-organisms *Bact Rev* 9, 1-47
- 61 REDDISH, G. F. (1957) Ed. by, *Antiseptics, disinfectants, fungicides and chemical and physical sterilisation* 2nd. Ed. Henry Kimpton, London.
- 62 RICE, H. M. (1958) Testing of air filters for hospital sterilisers *Lancet* 2, 1275-1277
- 63 RICE, H. M. (1960) A standard cartridge-form cottonwool air filter for pressure steam sterilisers *Lancet*, 1, 960
- 64 ROYCE, A. (1959) Modern sterilising and aseptic techniques *Publ Pharm* 16, 235-241
- 65 SAVAGE, R. M. (1937) Experiments on the sterilising effects of mixtures of air and steam and of superheated steam *Quart J Pharm* 10, 451-462
- 66 SAVAGE, R. M. (1954) The sterilisation of surgical dressings *J appl Bact* 17, 278-285
- 67 SAVAGE, R. M. (1959) Principles underlying steam sterilisation *Symposium on the operation of sterilising autoclaves* The Pharmaceutical press, London. 1-11
- 68 SAVAGE, R. M. and CHAMBERS, S. W. P. (1942) The sterilisation of tulle and paraffin surgical dressings *Quart J Pharm* 15, 291-300
- 69 SCHOU, S. A. (1950) Comparison between chemical and bacteriological effect of heat sterilisation. *Acta Pharm Intern* 1, 117-126
- 70 SCOTT, A. C. (1957) Gravity air-displacement pressure steam sterilisers Simple repair of some common faults *Lancet* 2, 633-637
- 71 SHOTTON, E. (1959) Processes of heat sterilisation. *Pharm J* 128, 315-317
- 72 SHOTTON, E. (1961) Problems of sterilising surgical materials with particular reference to thermal methods *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London. 1-6
- 73 SHOTTON, E. and SIMONS, F. M. (1950) The sterilisation of sulphonamides powders *J Pharm Pharmacol* 2, 231-235
- 74 SWIFT, H. B. (1950) A note on bactericides in solutions for injection *J Pharm Pharmacol* 2, 101-104
- 75 SYKES, C. H. (1958) Sterile dispensing and the hospital pharmacist. *M & B Pharmaceutical Bulletin* 7, 38-41

- 76 SYKES, G (1958) *Disinfection and sterilisation* E & F N Spon Ltd London
- 77 THIEL, C C, BURTON, H and MCCLEMONT, J (1952) Some aspects of the design and operation of vertical laboratory autoclaves *Proc Soc appl Bact* 15, 53-76
- 78 THOMPSON, R E M (1959) Cardboard boxes in operating theatres *Lancet* 1, 1150
- 79 THOMPSON, R E. M and O'GRADY, F W (1959) An improved box for the sterilisation of dressings *Lancet*, 2, 445-446
- 80 TODD, J P and SMITH, H L (1932) The bactericidal action of some common medicaments *Pharm J* 74, 185-186
- 81 TRAIN, D (1960) Heat sterilisation processes *Pharm J* 130, 195-196
- 82 WALTER, C W (1948) *The aseptic treatment of wounds* Macmillan, New York
- 83 WELCH, J D (1961) The organisation of central sterile supply departments *J clin Path*, 14, 69-75
- 84 WHITMARSH, J M (1958) *British Fermentation Industries* 1st Ed Pitman, London
- 85 WIEN, R (1939) The toxicity of parachloro-metacresol and phenylmercuric nitrate *Quart J Pharm* 12, 212-229
- 86 WILKINSON, G R (1959) The design of pressure steam sterilisers *Symposium on the operation of sterilising autoclaves* The Pharmaceutical Press, London 11-22
- 87 WILKINSON, G R (1960) Steam sterilisation. *Mfg Chem* 31, 427-433
- 88 WILKINSON, G R and PEACOCK, F G (1961a) The removal of air during autoclave sterilisation of fabrics using low pressure steam *J Pharm Pharmacol* 13, 67-71T
- 89 WILKINSON, G R and PEACOCK, F G (1961b) Improvement of heating of bottled fluids during autoclave sterilisation using low pressure steam ibid 72-74T
- 90 WILKINSON, G R and PEACOCK, F G (1962) Thermocouples for autoclaves *Lancet* 1, 488
- 91 WILKINSON, G R, PEACOCK, F G and ROBINS, E L (1960) A shorter sterilising cycle for solutions heated in an autoclave *J Pharm Pharmacol* 12, 197-202
- 92 WOOD, J H and TULLEY, J (1945) Temperature measurements in sterilisation *Pharm J* 154, 237-239

## The Preparation of Heat-Sterilised Injections

THE correct attitude to the dispensing of injections should not be in doubt after the following facts have been considered carefully

- 1 Injections are only given to patients who have genuine and, often, urgent need for them
- 2 Frequently, the active ingredients are potent and, therefore, an overdose might seriously harm the patient, while a deficiency could be equally disastrous through failure to produce the required effect
- 3 Sometimes medicaments are unstable and the omission of a stabilising agent might deprive the recipient of part, if not all, of the medication.
- 4 The patient's medical attendant will expect the preparation to be—
  - (i) Correctly, completely, clearly and cleanly labelled
  - (ii) Free from extraneous particles
  - (iii) Unquestionably fit for injection in its general appearance, i.e. the glass must sparkle, the sealing must be elegant and the packaging impeccable
- 5 Sterilisation should be assisted in every way possible, e.g. by keeping contamination low

*briefly, very high standards of accuracy, cleanliness and tidiness are essential*

### GENERAL ADVICE

- 1 Wear a freshly laundered long white coat or gown. It should be free from holes and, if of cotton, well starched. Nylon sheds fewer particles and is easier to launder
- 2 Work on absolutely clean surfaces. Dust should not be flicked into the atmosphere immediately before dispensing. Cleaning should take place some time earlier except, perhaps, in the case of plastic surfaces which can be wiped over with a damp cloth very shortly before use

3 Carefully check the balance which, also, should have been cleaned earlier. Adjust it if necessary and then put a sheet of clean white demy under each pan to facilitate removal of material accidentally spilled

4 Carefully read the prescription and, if relevant, the appropriate official monograph. Look for special requirements, e.g. an antioxidant or special stabiliser. From the method of sterilisation and the type of injection (single or multi-dose) decide if a bactericide is necessary, make a suitable choice, remembering the incompatibilities

5 Check every dose. Report overdoses and failure to prescribe the total amount, for a Dangerous Drug, or the dose, for a Schedule 4A poison. (Note that for these two classes of poison the Pharmacopoeia cannot follow the practice adopted for other injections, of recommending a strength when none is prescribed because of the legal obligation for the above information to be on the prescription.)

6 Copy out the formula and include in it any stabiliser or antibacterial agent that is necessary. Leave sufficient space at the right-hand side for the amounts actually used

7 Calculate the amounts needed and write them at the side. Check each one carefully

8 Write the labels and put them in a clean but conspicuous place at the back of the bench. Take care not to miss any special labels required by the monograph (e.g. instructions for dilution, administration or storage)

9 Put away, in a rack or drawer, the reference books you have used. While you are dispensing the only items on your bench, apart from equipment in use, should be the labels, prescription and calculation book.

10 See that the necessary containers and other items of glassware are scrupulously clean and dry. Cleaning should not be attempted at this stage, get replacements if necessary

11 Tidily arrange the essential equipment and when it is not in use keep it at the back of the bench or on a shelf

12 Since it is useful, on occasions, to invert measures and put down large stoppers, it is con-

venient to have a clean glazed-porcelain or glass tile at one side of the bench. This is not intended as the only working surface (attempts to use it as such are often seen) but to provide a particularly clean area for special purposes

## CALCULATIONS

Calculation errors can be avoided only by constant vigilance but they are less likely if the following rules are practised constantly

### 1 The formula is set out as recommended above

For example, for 10 ml of Ouabain Injection B P 1958

	B P Amounts	Amounts used
Ouabain	0 25 mg	2 5 mg
Water for Injection to	1 ml	to 10 ml

The proximity and arrangement of the two sets of figures facilitates quick comparison. If, by mistake, 2 mg is written instead of 2 5 mg its detection is more likely than if a separate formula for the amounts used is written underneath the B P amounts

### 2 The calculation is checked in more than one way

In the above example two possible methods are—

- (a) 10 is ten times 1  
Is 2 5 ten times 0 25?
- (b) 1 is four times 0 25  
Is 10 four times 2 5?

There is little chance of an error being overlooked if this practice is adopted

In some cases method (b) is not quite so easy to use, e.g. Mersaly Injection B P contains 9 56 G of mersaly acid, and other ingredients, in Water for Injection to 100 ml, but even with inconvenient amounts an approximate double check is usually possible and this will certainly detect gross errors in the amounts used column. In the example given, 9 56 is approximately 1/10 of 100, and the amounts used should be in the same ratio

### 3 The decimal point is always preceded by a nought

It is easy to write a decimal point so lightly that it is missed on re reading. In the ouabain formula, above, if the B P amount of ouabain had been written as 25 mg with the decimal point so faint that it was not seen when the amounts used were calculated, an attempt to dispense  $10 \times 25$  (250) mg in 10 ml might have been made. Fortunately, in this case, the low solubility of the drug would expose the mistake, but if it was possible to make a solution

of this strength it would contain 100 times the maximum dose per ml

It can be argued that even if the nought is used it is still possible to miss a faint decimal point and then only 1/10 of the prescribed dose might be dispensed (i.e. if 0 25 was interpreted as 025). However, this is unlikely because there will be a space between the 0 and the rest of the figures (e.g. 0 25) that will betray the missing point and, more important, since the use of the nought is now a recommended procedure, its appearance without a succeeding decimal point at the beginning of a number would be regarded with suspicion. Consequently, if it was not obvious that the point should be present, steps would be taken to confirm the dose. Since the use of the nought is not a complete solution it is equally important to see that the decimal point is clearly written

### 4 Figures are never altered

No attempt should be made to alter one figure to another. Although, often, the new figure is quite legible and completely obscures the one underneath, there are occasions when it is possible to read both and impossible to decide which is on top. The safest method is to strike out the group of figures to which it belongs and write the correction at the side or above. Occasionally, when only the wrong figure is crossed through it is done in a way that adds to the confusion. As an example, if, in the Mersaly Injection formula, 9 46 had been written instead of 9 56, 9 46 would be crossed through—not just the 4

### 5 Use the largest possible volumes in triturations

e.g. Adrenaline Injection B P Send 20 ml

	B P Amounts	Amounts used
Adrenaline acid tartrate	0 18 G	0 036 G
Sodium metabisulphite	0 1 G	0 020 G
Sodium chloride	0 8 G	0 160 G
Chlorocresol 0 1 per cent w/v in Water for Injection	to 100 ml	to 20 ml

36 mg of adrenaline acid tartrate is required

Although 50 mg is the minimum that may be weighed, 60 is more convenient because 6 is a factor of both it and 36. Moreover, this is only a small and, therefore, permissible excess.

An unsuitable trituration would be to dissolve 60 mg in sufficient chlorocresol solution to give a volume of 2 ml and then take 1.2 ml (equivalent to 36 mg) because these small volumes are difficult to measure accurately with conventional pharmaceutical measures (but see p. 384). A satisfactory alternative is to dissolve 60 mg in sufficient solvent to produce 20 ml and take 12 ml ( $\equiv$  36 mg).

Similarly for the metabisulphite, 50 mg should be dissolved in sufficient solvent to produce 15 ml and 6 ml ( $\equiv$  20 mg) taken.

However, when several triturations have to be made care must be taken to ensure that the sum of the triturations is not greater than the total volume of the injection.

#### *6 Give the complete procedure for making the final solution*

Errors are less likely if this is set down clearly before dispensing begins.

E.g. for 20 ml of Adrenaline Injection, underneath the formula should be written—

#### *Calculation*

- (i) Adrenaline acid tartrate 60 mg  
Chlorocresol solution to  $\frac{20 \text{ ml}}{12 \text{ ml}}$  ( $\equiv$  36 mg) taken.
- (ii) Sodium metabisulphite 50 mg  
Chlorocresol solution to  $\frac{15 \text{ ml}}{6 \text{ ml}}$  ( $\equiv$  20 mg) taken.
- (iii) Adrenaline and metabisulphite solutions mixed and 160 mg sodium chloride dissolved in the mixture.
- (iv) Volume made up to 20 ml with chlorocresol solution.

#### *7 Have the weights of poisons checked*

A full check involves not only the weighing and the drug but, also, the calculation. This is particularly important when injections containing poisons are being dispensed.

After reading this chapter attempt the calculations at the end.

### LABELLING

The patient's safety is particularly dependent on the label of an injection. Great care should be taken to see that it is—

*1 Accurate* This is ensured by checking it fully after writing and again just before fixing it on the container. The name of the injection and the strength are especially important. A final check by another person just before issue is desirable.

*2 Complete* The requirements of the B.P. or B.P.C. and where appropriate, the Poisons Rules, Dangerous Drug Regulations and Therapeutic Substances Regulations must be satisfied. The date of manufacture and a reference number are desirable in case a query arises at a later date. The official books sometimes require a special instruction on the label, e.g. restricting the route or giving details of dilution for use.

*3 Intelligible* The requirements must be arranged to avoid confusion and give prominence to the most important information.

*4 Legible* This is complicated by the smallness of some (e.g. ampoule) labels. It can be assured by avoiding decorative and flourishing handwriting and using bold, plain lettering and selective underlining. Handwritten labels should be done in permanent ink, preferably black because of its clarity. Washable ink smudges too easily during writing, while

fixing the label to the container, and in the hands of the user. Alterations if badly executed are a danger and, even if neatly done, give a bad impression. The label should be rewritten.

*5 Clean* Dirty and greasy finger marks are intolerable.

Flag labelling of ampoules (i.e. attaching a long label by one end and winding the rest round the ampoule) is not acceptable because part might be torn off. Information on an ampoule label is easier



Fig. 23.1

to read if it is written parallel to the long axis of the ampoule this should be taken into account when the size and shape of the label are being decided. In addition, since most people hold the neck of the ampoule in the right hand while looking at the label the latter should be fixed with its left-hand side towards the base of the ampoule (Fig 23.1). Ideally, the full length of the ampoule should be visible at the back to facilitate examination of the contents for signs of decomposition.

### THE INFORMATION ON THE LABEL

This is most conveniently discussed in relation to the type of injection.

#### A. Single-dose Injections of Large Volume

##### 1 BP or BPC Requirements

(a) General These will be found in the general sections on injections in the two books. They should also be used for guidance when non official injections are dispensed.

- (i) The name of the injection
- (ii) The strength For this type of injection it is usually required as percentage w/v. Sometimes, when the formula is complicated and the preparation well known, the statement of strength is waived, in such cases it is important to include the accepted abbreviation of the official source (B P, B P C etc) after the name, thus leaving no doubt that the injection is of official strength, e.g. Compound Sodium Lactate Injection B P. To an increasing extent the quantities of ingredients in infusion fluids are being expressed in milliequivalents, see, for example, the B P monographs for Potassium Chloride, Sodium Bicarbonate, Sodium Chloride, Sodium Lactate and Compound Sodium Lactate Injections.

(b) Specific For some injections the B P and B P C give definite special labelling instructions (in addition to the general requirements). For others the need for additional directions can be inferred from statements in the monographs. As an example of the latter the B P requires Dextrose injection to be stored in a cool place; this can only be ensured by appropriate labelling. The same applies to the statement about glass spicules in the monographs of the saline-containing infusion fluids.

##### 2 Dangerous Drug Act and 3 Pharmacy and Poisons Act Requirements

These are not relevant in this case because poisons, like bactericides, cannot be used safely in large volume injections.

#### 4 Other Requirements

Common sense dictates that the following information should be given—

- (i) An indication that the preparation has been sterilised, together with the date of sterilisation and, if several batches were sterilised on that date, a reference number. The inclusion of the date and number is to allow details to be traced in the production record later e.g. Sterilised 12.3.63 (3)
- (ii) The patient's name—if the preparation is supplied on a prescription
- (iii) The total volume—it is useful to the user to know this
- (iv) The name and address of the hospital or manufacturer—normally already printed on the label!

#### B Single-dose Injections of Small Volume

Two labels must be considered in this case, one for the container, which is most often an ampoule, and the other for the protective package, which is usually a box holding several containers.

##### ON THE AMPOULE

##### BP or BPC Requirements

- (i) Name of the injection
- (ii) Strength—usually expressed as the amount of drug in the nominal volume, i.e. the volume that the user expects to remove—e.g. 1 ml from a 1 ml ampoule. It is much more convenient for the user to have small amounts expressed in milligrams or micrograms and not in fractions of a gramme, e.g. 3 mg not 0.003 G and 600 micrograms (mcg), not 0.0006 G. Occasionally, percentage w/v or 1 part in so many parts of solvent is used. Rarely, the letters B P or B P C after the name are sufficient (see above).

##### ON THE BOX

##### 1 BP or BPC Requirements

- (i) and (ii) as for ampoule
- (iii) Name and proportion of any bactericide—this applies if the sterilisation method was Heating with a Bactericide
- (iv) Special instructions These may include directions for dilution, an instruction to protect from light, and a warning to use a particular route for injection, also, the nature of buffering, dispersing and stabilising agents and the composition of oily vehicles may be required.

## **2 Dangerous Drug Act Requirements**

If the injection is a Dangerous Drug the number of ampoules in the box must be stated. The amount in each, which is also required, is covered by 1(ii)

## **3 Pharmacy and Poisons Act Requirements**

- (i) If the injection is a Part 1 or Part 2 poison the word 'Poison' is required and this must be in red or on a red background if the injection is in Schedule 1

If the injection is affected by the first section of Schedule 7 the words prescribed in this section, i.e. Caution, it is dangerous to take this preparation except under medical supervision' must be used instead of 'Poison'.

Since the label is certain to contain words other than those required by the Act, if it contains all the information recommended here, the word 'Poison' or other alternative words must be surrounded by a line or be on a separate label

- (ii) The name of the seller and the address of the premises from which the injection was sold or supplied.

## **4 Other Requirements**

- (i) 'Sterilised', the date and the batch number
- (ii) The patient's name, if relevant
- (iii) The name and address of the hospital or manufacturer, if not covered by 3(ii)
- (iv) Even if the preparation is not a poison or dangerous drug it is desirable to state the number of ampoules

If the injection is supplied on the prescription of a medical practitioner sections 2 and 3(i) above do not apply but when supply is on the prescription of a dentist or veterinary surgeon only section 3(i) is not applicable

## **C. Multiple-dose Injections**

### **1 B.P. or B.P.C. Requirements**

- (i) Name of the injection
- (ii) Strength—usually expressed as the amount in a suitable dose volume. Occasionally the modifications mentioned under B are required instead
- (iii) Name and proportion of any bactericide used as a preservative or in connexion with Heating with a Bactericide
- (iv) Special instructions—see B1(iv) above

### **2 Dangerous Drug Act Requirements**

- (i) The total amount of the preparation in the container (i.e. the volume in the case of fluids for injection)

- (ii) The percentage of dangerous drug in the preparation N.B. this is required in addition to 1(ii) unless the strength has been stated as a percentage

## **3 Pharmacy and Poisons Act Requirements**

- (i) 'Poison' etc—see B3(i)
- (ii) Name and address of the seller—see B3(ii)

## **4 Other Requirements**

- (i) 'Sterilised', the date and batch number
- (ii) The patient's name, if relevant

Even if the preparation is not a poison or dangerous drug it is desirable to have the requirements of 2(i) and 3(ii) on the label

If the injection is supplied on the prescription of a medical practitioner 2(i) and (ii) and 3(i) do not apply but if supply is on the prescription of a dentist or veterinary surgeon only 3(i) is not applicable,

## **Supplies from Hospitals**

- (a) *To outpatients* The above requirements, as for supply on a prescription, apply
- (b) *To inpatients* The Pharmacy and Poisons Act requirements listed above for the ampoule box and multiple dose container do not apply but the Dangerous Drug Act requirements do. An identifying mark is necessary on the label of a dangerous drug and a Schedule I poison to show that it must be stored in the ward dangerous drug or poisons cupboard respectively. The letters D.D. and S.I. respectively, would be suitable, there is no legal recommendation

An attempt has been made to state the legal requirements as briefly as possible, for further information reference should be made to a textbook of forensic pharmacy (e.g. Fowler, 1960)

The labelling requirements of the Therapeutic Substances Act chiefly affect injections that are not heat sterilised (dimercaprol, posterior pituitary hormone and tubocurarine injections are exceptions) and will be considered in chapter 26 where this type of injection is discussed

## **SPECIMEN LABELS**

### **1 Single-dose Injections of Large Volume**

Note that throughout these labels only the name of the injection is both in capitals and underlined. Consequently it is more prominent than any other item.

The B.P. requires a statement of the strength in mEq when Sodium Chloride Injection is intended for intravenous infusion

500 ml

SODIUM CHLORIDE INJECTION B P

0.9% w/v

154 mEq of sodium and chloride ions per litre

Warning On storage this injection may cause small solid particles to separate from the container A solution containing such particles must not be used

Sterilised 12 3 65 (3) Name

(b) PREPARED BY HEATING WITH A BACTERICIDE  
(*Not a poison or a dangerous drug*)

*Ampoule*

SODIUM
AUROTHIOMALEATE
INJECTION B P
10 mg in 1 ml

*Box*

PROTECT FROM LIGHT
--------------------

Name' refers to the patient The space at the bottom of the label is for the name and address of the manufacturer or hospital

The warning about glass spicules is long and could be on a separate label but if practicable it is better to use a label large enough to take all the information because then there is less chance of anything being overlooked by the user For the same reason if additional labels are preferred they should not be put at the back or the bottom front of the bottle The warning that any remainder must be thrown away (see p 260) should be on the container of this type of injection

**2 Single-dose Injections of Small Volume**

(a) PREPARED BY AUTOCLAVING (*Not a poison or a dangerous drug*)

*Ampoule*

NIKETHAMIDE
INJECTION B P
1 G in 4 ml

*Box*

Six Ampoules

NIKETHAMIDE INJECTION B P

1 G in 4 ml

Sterilised 12 3 65 (3) Name

Six Ampoules

SODIUM AUROTHIOMALEATE  
INJECTION B P

10 mg in 1 ml

Bactericide 0.2% w/v Chlorocresol

Sterilised 9 4 65 (1) Name

The word bactericide has been underlined (but not put in capitals) to break up the information in the lower part of the label Excessive underlining should be avoided

(c) PREPARED BY AUTOCLAVING  
(*Schedule 1 poison not supplied on a prescription*)

*Ampoule*

ANTIMONY SODIUM
TARTRATE INJECTION
B P
60 mg in 1 ml

Box

Six Ampoules

ANTIMONY SODIUM TARTRATE  
INJECTION B.P.

60 mg in 1 ml

**POISON**

Sterilised 17 5 65 (5) Name

The word Poison would be in red  
*If supplied on a prescription or to a hospital out patient* Poison' would not be necessary, for a hospital in patient it would be replaced by a distinguishing mark to indicate that storage in the ward poisons cupboard is necessary

(d) PREPARED BY HEATING WITH A BACTERICIDE  
*(Dangerous drug not supplied on a prescription)*

Ampoule

MORPHINE SULPHATE  
INJECTION B.P.  
20 mg in 1 ml

Box

**PROTECT FROM LIGHT**

Six Ampoules

MORPHINE SULPHATE  
INJECTION B.P.

20 mg in 1 ml

**POISON**Bactericide 0.2% w/v Chlorocresol

Sterilised 4 6 65 (1) Name

The comments under (c) apply except that the mark on the label for a hospital inpatient would indicate storage in the ward Dangerous Drug cupboard

## 3 Multiple-dose Injections

(a) PREPARED BY AUTOCLAVING (*Not a poison or a dangerous drug*)

**PROTECT FROM LIGHT**

Shake the Bottle

30 ml

BISMUTH OXYCHLORIDE  
INJECTION B.P.C.

0.2 G in 2 ml dose

For intramuscular injection only

Bactericide 0.1% w/v Chlorocresol

Sterilised 21 7 65 (1) Name

(b) PREPARED BY AUTOCLAVING (*Poison, but not Schedule 1 not supplied on a prescription*)

**PROTECT FROM LIGHT**

10 ml

HYOSCINE INJECTION B.P.

400 micrograms in 1 ml dose

**POISON**Bactericide 0.1% w/v Chlorocresol

Sterilised 21 7 65 (1) Name

If supplied on a prescription or to a hospital in or out patient the word 'Poison' is not necessary

(c) PREPARED BY HEATING WITH A BACTERICIDE  
(*Dangerous Drug, not supplied on a prescription*)

20 ml

**PAPAVERETUM INJECTION B P C**

20 mg papaveretum, equivalent to about 10 mg (1% w/v) anhydrous morphine in 1 ml dose

**POISON**

Bactericide 0.002% w/v Phenylmercuric Nitrate

Sterilised 27 8 65

Name

5 They are apt to separate from containers stored in a refrigerator

A superior method is to print directly on to the glass, and suitable equipment is made by Rejafix. The type, or a ready made block, is assembled in the machine and, after automatic inking, is pressed on to a soft pad. Then the container is rolled over the wet impression. Two inks are available, one dries in air but is comparatively easily removed by cleaning processes and after immersion in certain disinfectants. The other must be baked on at about 500°C but is then indelible. An important advantage of this technique is that the contents of the container are clearly visible. If repeatedly-used bottles are glass printed they must never be filled with a different injection and paper-labelled over the printing because of the serious results that might follow if the paper label became detached.

Printed self adhesive labels are obtainable for a few common sterile products, such as Water for Injection and Sodium Chloride Injection. Although the printing is exceptionally clear and the surface dirt repellent, the edges attract dust quickly and, since they are usually supplied in a roll, care is required to cut them neatly.

If ampoules are stored in toxic bactericides the latter may enter through invisible cracks in the glass (Bean and Walters, 1954) and cause injury when the injection is administered. This can happen even from quite strongly coloured solutions without the leakage becoming detectable (Cope, Prescott and Whittet, 1952). Storage of a spinal anaesthetic in 5 per cent phenol led to paralysis from the waist down of two patients (Case Report, 1953). The danger to the patient from bacterial contamination of the outside of an ampoule is much smaller and, consequently, the use of storage solutions should be condemned. As alternatives, thermostable injections might be glass printed and packed singly in labelled heat sealed paper bags before steam sterilisation, while treatment with ethylene oxide gas (q.v.) might be suitable for similarly packed thermolabile injections.

(For general articles on labelling see Shepley (1950) and Patel (1955))

### CLEANING CONTAINERS AND GLASSWARE

No particular routine for cleaning glassware can be claimed to be superior to all others, there are many opportunities for variation and choice is largely influenced by personal experience (e.g. see Cooper,

1951, Fowler, 1959, Myers, 1948). Nevertheless a brief consideration of the aims, principles and general procedure is justified by the importance of scrupulously clean glassware for parenteral solutions.

**Aims**

The container must be—

1 *Chemically clean*, i.e. all traces of cleaning agents, as well as dirt, must be removed

2 *Free from particulate matter* Contamination of a carefully filtered injection with fibres and other small particles from the container can considerably increase the number of bottles rejected on final inspection.

3 *Free from pyrogens*, because the official sterilisation methods cannot be relied upon to destroy them.

4 *Free from grease*, see Sterilisation Methods

**Principles**

1 Used containers should not be allowed to dry, because the resulting film of medicament may be hard to remove

2 For speed and efficiency, mechanical apparatus should be used where possible, e.g. high-speed bottle brushes and multi-jet rinsers

3 Potentially damaging practices must be avoided, e.g. the use of abrasives or strongly alkaline cleaning agents. Bottle necks may be chipped by the metal mounts of brushes and the nozzles of rinsers but some protection can be given by covering the metal with rubber tubing. Similar covers over the metal tips of bottle brushes prevents the bottoms of bottles from becoming scratched

4 The cleaning agent must be chosen carefully—

(a) *Organic detergents* These may be strongly adsorbed on to glass, because of their high surface activity. Vigorous rinsing is needed to prevent a residual film that might be desorbed during sterilisation. For this reason, cationic detergents, some of which are strongly antibacterial, must not be used for bacteriological glassware. The use of excessive amounts of any detergent is unwise. They are satisfactory in hard and soft water.

(b) *Soap flakes and powders* A serious disadvantage of these is the precipitation of calcium and magnesium soaps that occurs in hard water, particularly as the precipitate deposits on glass surfaces, impairing their brilliance. Use of a water softener such as sodium hexametaphosphate eliminates this problem.

(c) *Inorganic detergents* (see Resuggan, 1957), e.g. sodium metasilicate and sodium hexametaphosphate. The latter reacts with the calcium and magnesium salts of hard water to give very soluble metaphosphates that are not precipitated by soaps. Glassware washed in solutions of it, alone or with soap, has a

strikingly brilliant appearance. Inorganic, unlike organic, detergents are easy to rinse from glass surfaces.

(d) *Chromic acid* A popular cleaning solution is made by dissolving about 70 G of sodium or potassium dichromate in about 40 ml of water, using heat, and then, after cooling, making up to 1 litre with concentrated sulphuric acid. Addition must be slow and with constant stirring, and the solution must never be added to the acid. Great care is necessary when preparing and handling this reagent, gloves and a rubber apron should be worn to protect the skin and clothes (especially nylons!). It has two main applications, the removal of difficult stains, for which storage overnight in the acid is desirable, and the destruction of pyrogen films. The latter are not produced if a good cleaning routine is used, but when occasional precautionary treatments are thought desirable a few minutes exposure should be sufficient. Its use must be followed by thorough rinsing and checks for residual acidity and chromate ion, because a large number of medicaments are destroyed by oxidation.

5 Wet containers must not be dried neck uppermost at room temperature because during slow drying, bacterial growth and pyrogen production can take place in the films of water in the bottoms of the containers. Dry pyrogen films are most difficult to inactivate, dry heat exposures of 250°C for 40 min or 200°C for at least 2 hr being necessary.

**General Procedure**

1 *Soaking* The containers are immediately filled with, and immersed in, hot cleaning solution, and, preferably, left overnight.

*2 Brushing*

3 *Rinsing* First, hot and cold tap water are used. Both inside and outside of the container are rinsed at the same time, if this is not done, cleaning solution that runs down the neck on the outside, while the bottle is inverted, may enter the mouth when the bottle is reverted (see Fowler, 1959). Afterwards, final rinses are given with freshly distilled apyrogenic water, continuous rinsing is not practicable in this case because of the cost of the water but the containers should be filled and emptied at least three times.

4 *Drying* After draining, the containers are dried in a drying oven or cabinet. This is to eliminate the small inaccuracy that would result if the final solution was filled into a wet bottle. It also allows a more thorough inspection of the glass for dirty marks.

It is not easy to protect a container from dust particles during drying even when it is inverted with

its neck clear of the shelves and its mouth tightly covered with a smooth water vapour permeable paper. Therefore, it is not uncommon for large bottles to be used immediately after cleaning, they are well-drained but, inevitably, slightly wet.

Containers not required at once may be partly filled with freshly distilled water, capped and sterilised, this procedure provides an opportunity to check the water, in one or two bottles, for excess alkali or traces of cleaning acid but it sometimes makes the rubber closures more prone to shed particles in the subsequent sterilisation of the injection.

Small containers should always be dried because the dilution caused by residual moisture could be significant.

### CLEANING OF CLOSURES

#### (a) ALUMINIUM CAPS OF TRANSFUSION BOTTLES

Boil for 10 min in weak detergent solution

If necessary, remove resistant stains by immersion in warm sodium carbonate solution (5%) for 5 min. Longer may lead to significant solution of the aluminium.

Wash thoroughly in running hot water. A glass tube connected to the tap and reaching to the bottom of the vessel is helpful.

Boil in tap water for 15 min. Rinse well.

Boil in distilled water for 15 min.

Rinse three times in distilled water.

Dry, top uppermost, on a clean sheet of glass in a warm oven.

Store in closed containers, e.g. biscuit tins.

#### (b) BAKELITE CAPS FOR CLINBRITIC BOTTLES

As for aluminium caps but without the alkali treatment.

Use a warm, not a very hot, oven for drying.

#### (c) RUBBER LINERS, PLUGS AND CAPS

Anxiety to free rubber from all extractable matter must not encourage the use of drastic treatments. Some of these can adversely affect the rubber compound, giving it unsatisfactory physical characteristics such as stickiness and causing it to liberate extractives that under normal conditions of use would have remained firmly bound. In addition, the ability of rubber to absorb substances from solution must not be forgotten because some cleaning agents, especially organic compounds, may be retained and, later, liberated into injections during sterilisation. These considerations suggest two rules for cleaning closures—

No glassware used for parenteral solutions should be dried with a glass cloth. It is instructive to dry a beaker in this way, fill it with freshly distilled water and examine the liquid.

*5 Inspection* The dried containers should be carefully inspected for dirty marks and any rejects sent back for recleaning, then chromic acid treatment will often be appropriate. Bottles with chipped necks are thrown away.

*6 Storage* The paper caps of the dried containers should be given an outer covering as protection against dust, and the bottles stored in a dust proof cupboard at an even temperature.

Tests for alkalinity must be carried out on new containers and on old containers at intervals during their life.

1 Solutions far removed in chemical nature and concentration from those used in injections should be avoided.

2 After cleaning the closures should be immersed in distilled water and subjected to a heat treatment at least as stringent as the sterilisation process in which they will be used. This will extract cleaning agents that otherwise might be liberated into injections.

The selection of a method is influenced by the composition of the rubber. If satisfactory results can be obtained only with great difficulty closures of a different composition should be tried. New articles may require one or more of the following treatments—

(i) Boiling in weak detergent. This is to remove mould lubricant, extractable colour and loose particles of dirt and rubber. The concentration of detergent should be well below 1 per cent.

(ii) Boiling in dilute alkali. This is primarily to remove sulphur compounds, particularly from the bloom. One per cent or less of sodium carbonate should be adequate.

(iii) Boiling in dilute acid. This is desirable because many injections are acid, and extracted alkali could affect their stability. It must always follow alkali treatment. One per cent of hydrochloric acid is suitable.

(iv) Boiling in a solution of a sequestering agent such as sodium hexametaphosphate or E D T A. In addition to their water-softening and detergent activities, these substances help to remove compounds of heavy metals such as zinc from the surface.

Commonly about 15 min boiling is used in each case, followed by vigorous washing in running hot water. Then, after boiling in distilled water and several rinses in the same solvent, the caps are sterilised. Finally, the sterilisation water is poured away and cleaning completed by three more rinses in distilled water. A plastic salad shaker is very useful for holding the closures between boilings and rinsings; there is no danger of the contents falling out, and thorough shaking is possible without risk of damage.

In hospitals, closures (except transfusion bottle plugs, which are not used more than twice) are often re-used. Less treatment is necessary than for new caps but a thorough routine is essential. Also, each closure must be carefully inspected for freedom from large holes at the perforation site, stickiness and roughness.

Clean transfusion bottle closures that are not used immediately can be either—

(a) Dried in a warm cupboard on a spotlessly

clean glass sheet covered, at a distance from the closures, by a large sheet of smooth paper, e.g. cellulose film. Alternatively, a beaker may be used but drying is slower. Afterwards they can be stored in a clean, dry, screw-capped jar. Or

(b) Autoclaved with distilled water in a screw-capped jar. Because bactericides are forbidden in infusion fluids they should not be used in storage solutions, although it is rather unlikely that a harmful amount would be leached from a closure. When water alone is used sterilisation is essential to prevent bacterial multiplication and pyrogen production, and if all the closures are not used when the container is opened the rest must be resterilised.

Closures for multiple-dose containers of injections containing a bactericide must be equilibrated in an appropriate solution by the method outlined in chapter 21. Closures for injections containing no bactericide are autoclaved in water as in (b) above.

All rubber closures should be stored in the dark.

## THE QUALITY OF MEDICAMENTS

All injection medicaments must be of high chemical purity and not contaminated with dirt, fibres and other foreign matter from faulty packaging and careless storage. In addition, freedom from pyrogens is preferable in all cases and essential for the ingredients of infusion fluids and for substances, such as calcium gluconate, of which large doses are given intravenously although in relatively small volumes.

The care taken to remove and exclude pyrogens in the preparation of Water for Injection is wasted if the medicament is pyrogenic. The substances most likely to be unsatisfactory (Whittet, 1954) are fermentation products, since these are obtained from micro-organisms, and animal products, because the extracts and extraction temperatures sometimes provide excellent conditions for the multiplication of bacterial contaminants. Fermentation products that must comply with a test for pyrogens include antibiotics (benzylpenicillin, streptomycin, tetracycline and viomycin), salts of organic acids (calcium gluconate and sodium lactate) and dextran. Animal products are represented by chorionic gonadotrophin, heparin, hyaluronidase and protamine sulphate.

Some dry chemicals, originally satisfactory, become pyrogenic from bacterial contamination during storage and, therefore, watchfulness is necessary even with substances such as dextrose, sodium chloride and sodium bicarbonate that are usually pyrogen-free after manufacture.

The problem can be attacked in several ways—

1 Samples from the completed batch of injections can be tested for pyrogenicity and, if they fail, the whole batch can be discarded. This method is only practicable if the medicament is cheap, contamination with pyrogens is unlikely and testing facilities are readily available. The last is not the case in most hospitals.

2 Adsorption methods may be used to remove pyrogens from the injection solution—

(a) The solution is shaken with activated charcoal (see the B.P.C. monograph for charcoal). Brindle and Rugby (1946) suggested intermittent shaking for 15 min with 0.1 per cent, and Hudson (1944) developed a technique for the continuous treatment of infusion fluids. The charcoal must be free from soluble impurities and particles too fine to be retained by a clarifying filter. A little medicament is adsorbed but since this is only about 30 per cent of the weight of the charcoal it is negligible in infusion fluids.

(b) The solution may be passed through a pyrogen-retaining asbestos and cellulose filter pad. The Ford Sterimat GS/PYR (Ford, 1960) is claimed to be suitable. If the pyrogen content is high some may pass through because the method of removal is by adsorption of a solute, not retention of insoluble particles. Efficiency can be improved by increasing the adsorption surface either by using a thicker pad.

or several pads in series. Significant adsorption of the medicament may take place if its concentration is small, as in the case of heparin injection (see Bacterial Filtration).

3 The most common practice is to rely on the supply of apyrogenic drugs by manufacturers. Sometimes satisfactory chemicals are obtained by normal production methods, for example, Davis (1946), in 10 years as a hospital pharmacist, received no complaints of pyrogenic reactions from injections made with anhydrous dextrose of pharmaceutical quality. In other cases it may be possible to avoid pyrogenicity by carrying out much of the production at temperatures unfavourable to bacterial growth, or to include antibacterial agents (e.g. toluene in the digestion stage of the extraction of heparin) or, for costly drugs that are soluble in a manageable volume of water, to use a pyrogen retaining filter towards the end of the process. Where the B.P. requires the substance itself to be pyrogen-free (e.g. antibiotics, heparin and hyaluronidase) the purchaser can be sure that satisfactory material will be supplied, for other medicaments evidence of long reliability should

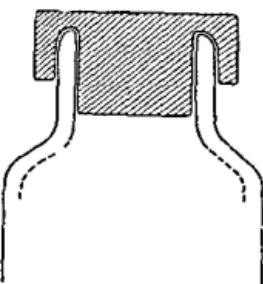


Fig. 23.2 DUST PROOF CONTAINER

be sought. Sodium chloride is exceptional because it can be made apyrogenic by heating almost to redness before use (Smith, 1954).

Drugs for injection should be received in well-sealed glass containers and stored in dust proof bottles, the latter have a stopper that overhangs the rim and prevents dust from collecting there (Fig. 23.2).

### PREPARATION OF THE SOLUTION

The following procedure is generally applicable—

1 Collect together all the necessary apparatus and ingredients, this is to minimise movement once dispensing has started.

2 Pour into a conical flask or beaker a suitable volume of solvent. Non sterilised freshly distilled apyrogenic water is satisfactory provided the injection is immediately sterilised.

3 Protect the solvent from atmospheric dust, a small inverted beaker is suitable for a flask and a stainless steel or glass 'clock-glass' for a beaker. Cellulose film discs are useful for covering the mouths of narrow measures, they do not shed fibres but must be held in position with an elastic band, otherwise they spring off at the slightest air movement.

4 Check the label and appearance of the medicament and carefully weigh the required amount. All poisons for injections, whatever their weight, must be weighed on a chemical balance, and the same applies to all but very large amounts of non poisons, although not dangerous by mouth some non poisons have powerful physiological effects in minute doses when given parenterally, e.g. histamine acid phosphate. Preferably, weigh on to stainless steel watch-glasses, once a pair have been counterbalanced

adjustment is less often necessary than with glass pans, which get clapped or broken. Hold the bottle near to horizontal to protect the contents from dirt and micro-organisms in the air and, if the stopper is too large to hold in the same way as a bacteriological closure (between the little finger and palm of the other hand) put it, top uppermost on the tile. Use a clean spatula and try to keep the medicament off the ground glass neck.

5 Check the weights and the drug and then, if the latter is a poison, have the weight checked.

6 Tip as much as possible of the powder into the solvent and wash in the rest. Do not tap the pan on glassware because small splinters of glass may be clapped off into the solution. Note that the medicament is added to the solvent, not vice-versa, this prevents the formation of a slowly soluble mass at the bottom of the container. Stir to dissolve, using an annealed glass rod from which fragments cannot separate. It is often necessary to apply gentle heat, this may be done on a small hot plate, preferably, or on a tripod and gauze over a low Bunsen flame. Often, water baths are too slow, and much time can be wasted trying to dissolve a fairly large amount of a substance like dextrose by this means. Mild heat is so frequently advantageous that it is always desirable to make the solution in a container that

can be heated, only rarely is it advisable to attempt preparation in a measure (It is an indelible experience to try to make 50 per cent dextrose injection by putting the dextrose in a 100-ml measure, adding water and stirring) Intermittent shaking or stirring is necessary during warming to avoid overheating of undissolved powder at the bottom

7. Weigh and dissolve any other ingredients and, after cooling, make up to a convenient volume for filtration. For large quantities it may be convenient to do this in stainless steel vessels but these are not

#### pH Adjustment

This has been discussed under the preparation of culture media and the formulation of injections and only one or two points of practical importance need be added—

1 To avoid altering the strength of the solution, adjustment should take place before making up to the final volume but since subsequent addition of solvent may slightly alter the pH the volume used for the adjustment should be as large as possible

2 Adjustments on future occasions are greatly facilitated if an adjusting solution of definite strength is used and the exact amount required for a particular volume of injection is recorded. For example, for adjusting small volumes of Bismuth Sodium Tartrate Injection a 1 per cent solution of tartaric acid is suitable

ideal for dissolving the medicament because it is not easy to see when solution is complete

The batch size is influenced by the sterilisation facilities Because of the readiness with which pyrogens are produced in solutions left at room temperature, batches must be small enough for sterilisation on the day of preparation, or facilities should be available for refrigerated storage overnight. The danger is greatest with solutions such as dextrose or sodium lactate in which micro-organisms can multiply quickly

#### of the Solution

3 Where the medicament is a salt, adjustment should be made with the corresponding acid or alkali, e.g. maleic acid for ergometrine maleate and sodium hydroxide for heparin (a sodium salt)

4 If capillaries are used care is necessary to avoid contaminating the bulk of the injection with the indicator or the indicator with the injection. The latter is easily done by inadvertently using the same capillary, first for the injection and then the indicator

5 With indicator papers the most reliable results are obtained when they are dipped in the solution but the bulk of the injection must not be used—a drop should be tested in a small watchglass

6 After making up to volume check the pH again preferably with a pH meter

### FILTRATION

A satisfactory filtering medium for injections must produce a particle-free solution at a fast rate and without significant adsorption of medicament.

#### I COTTON WOOL, FILTER PAPER AND MUSLIN

These materials tend to yield fibres to the filtrate and, although careful prewashing sometimes makes them quite satisfactory, they are unreliable in this respect. Sykes (1958), for sodium citrate solution, preferred a pad of muslin packed into the stem of a funnel, to a glass filter, but he emphasised the importance of correct packing density. The high absorbency of cotton wool leads to considerable swelling and consequent slowing of filtration, while with filter papers used in conical funnels there is a risk of fracture at the apex when large volumes are being filtered. All three have the following disadvantages (i) Care is necessary to prevent bypassing of the medium at the edges (ii) They may adsorb significant amounts of medicaments from small volumes of dilute solutions

(iii) It is unwise to use them for clarifying supersaturated solutions, such as Calcium Gluconate Injection, because detached particles can act as nuclei for crystallisation

#### 2 SINTERED GLASS

This is the most popular medium. Grades 3 and 4 with maximum pore sizes of 15 to 40 and 5 to 15 microns respectively (see B.S. 1752 1963) are used for clarification. Most solutions will pass through grade 3 by gravity, and a very clean filtrate results. However, gravity filtration of large volumes may take several hours, which is undesirable because of the possibility of pyrogen development meanwhile, assistance from vacuum below, or pressure on top of, the liquid is necessary. This should, nevertheless, be minimal otherwise fibres will pass through with the filtrate. Grade 4, although too slow for gravity filtration, is very satisfactory when aided.

Dics of the medium are fused into a variety of glass holders to produce pipeline, Buchner-funnel

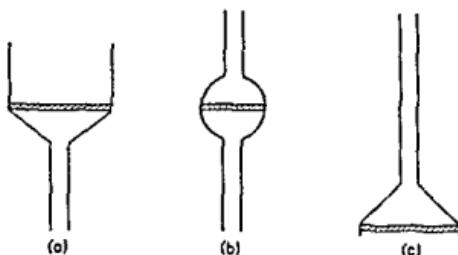


Fig. 233 TYPES OF SINTERED GLASS FILTER

(a) Buchner funnel (b) P p line (c) Immersion

and immersion type filters (Fig. 233). Generally, Buchner-funnel types are used for small volumes, and the other types for large. Contamination with dust during filtration is more likely with the funnel type, which must always be covered loosely, e.g. with a plastic watchglass.

Glass filters do not shed fibres and are easy to clean, and the solution cannot pass the medium. Their flow rates are good (grade 3) or satisfactory (grade 4), e.g. a grade 3 disc of 3 cm effective diameter, under a vacuum of 25 cm Hg passes about 200 ml/min, while the flow through a Grade 4 is about 1/6 of this.

### 3 SINTERED METAL

Durability is the outstanding advantage of this type. It consists of a sintered disc of stainless steel that can be sealed by a ring of silicone rubber into several different fittings to produce, for example, a Buchner funnel, pipeline or immersion filter (Fig. 234). The latter is used by inversion in the solution, and application of suction to the stem. The grades that

Table 23 I

Grade	Approximate mean pore size ( $\mu$ )	B S 1752
		Grade No
5	6.5	4
10	12	
20	18	
40	28	3
50	33	

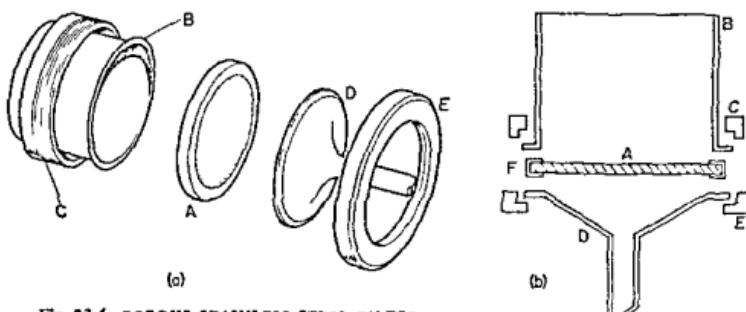
approximately cover the pore size ranges of sintered glass types 3 and 4 are given in Table 23 I.

The metal parts can be cleaned in 20 per cent caustic soda or 15 per cent nitric acid without affecting the pore size. Unlike sintered glass, where the opposite is the case, the disc with the finest pores is the strongest. Separate discs can be kept for different solutions to prevent contamination due to careless washing. The most unsatisfactory feature of sintered glass filters, the fragility of the holders, is not a problem with stainless steel types.

Because of the very large surface of metal within the sinters, they are liable to attack by corrosive solutions and, as a precaution, should be washed thoroughly, immediately after use, particularly when salines have been filtered. Newman (1954) reported very favourably on the suitability of these filters for intravenous fluids.

### 4 PLASTICS

One of the earliest plastics used for the filtration of parenteral solutions was polyvinylchloride but its use was discontinued because it liberated traces of lead impurity (Darwin and Dec, 1957). More recently

Fig. 234 POROUS STAINLESS STEEL FILTER  
(BUCHNER FUNNEL TYPE)(a) Parts exploded (b) The same, shown diagrammatically  
(Courtesy Adelphi Ltd.)

A—Sintered disc  
B to E—Body parts  
F—U-shaped silicone rubber sealing ring

much interest has been shown in certain cellulose esters, particularly cellulose acetate, and it seems certain that other useful materials, e.g. from high density polythene, will be developed in the future.

#### *Cellulose Esters*

These filters are of particular interest in sterilisation by filtration and sterility testing (q.v.) but because of their high flow rate, freedom from loose particles and low adsorption of medicament they are potentially useful for clarification. The American Millipore range includes a disc with a pore size of 5 microns which is approximately equivalent to a 4 sintered glass. As each membrane is used only once and their cost is fairly high they will not replace glass easily, at least for large volume injections.

#### 5 METAFILTRATION (See Cooper and Gunn, 1957)

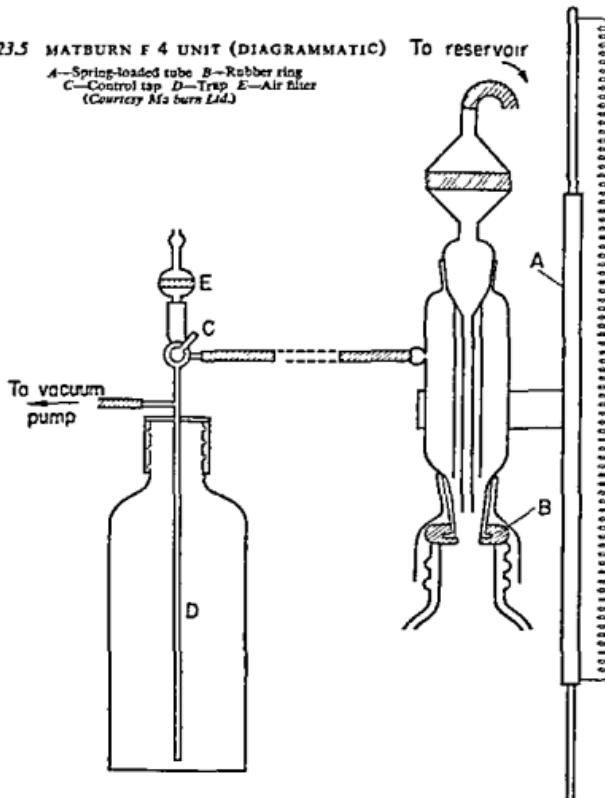
At least one hospital in this country uses this method for the clarification of intravenous fluids. Filtration is good and rapid but allowance must be made for the adsorption of medicament from the first part of the filtrate by the filter aid. It is unsuitable for small volumes.

#### Filtration Units

Usually filters are made part of a unit consisting of a container for unfiltered solution, the filter and a receiver for the filtrate. Often the receiver is so designed that the containers can be filled directly from it. Hospital pharmacists have devoted much attention to the construction of reliable apparatus.

Fig. 23.5 MATBURN F 4 UNIT (DIAGRAMMATIC) To reservoir

- A—Spring-loaded tube B—Rubber ring  
C—Control tap D—Trey E—Air filter  
(Courtesy Matburn Ltd.)



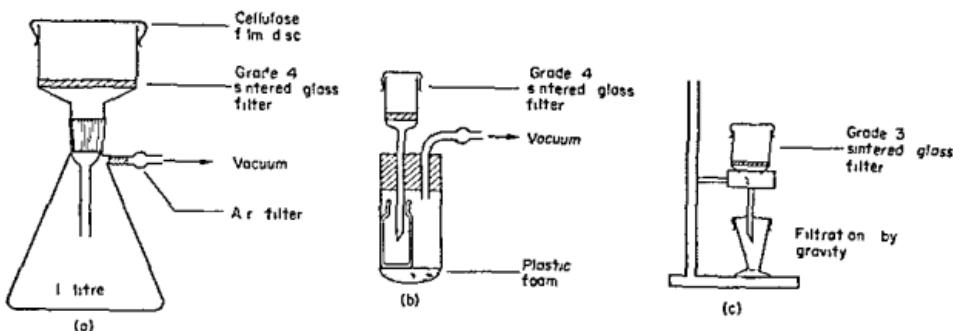


Fig 236 SIMPLE FILTRATION UNITS

and many descriptions have been published e.g., Cooper (1952), Etchells (1951), Gosby and Hanson (1950), Thom (1950). From these several conclusions can be drawn—

(a) A closed circuit is preferable, to reduce contamination from dust and bacteria

(b) Articles of natural rubber should not be used. For tubing, synthetics, such as medical grades of PVC or silicone are preferable, they are much cleaner, nevertheless the number and length of the pieces are kept to the minimum. A major disadvantage of many rubbers is the ease with which particles can be torn off by glassware, e.g. when the stem of a sintered glass funnel is pushed through a bung or a rubber tube is joined to a glass one. This can be reduced markedly by annealing all rough glass edges. Sometimes rubber can be replaced by a ground-glass joint.

(c) Glass and metal taps and ball valves give quicker and more sensitive flow control than screw or pinch clips, generally, pinch clips are unsuitable for vacuum tubing.

(d) A vacuum source is an asset for large volumes. A simple water jet pump is satisfactory if the water pressure is good but power-operated pumps are more reliable, and some types can also be used to supply compressed air. As the working parts of the most popular type of power pump operate in oil they must be protected from aqueous fluids accidentally sucked into the line during evacuation. Permanent installations usually have a moisture trap fitted at a low point in the piping and a tray of drying agent near to the pump, but a large flask and a calcium chloride tower are suitable alternatives in a more temporary set up.

An air filter is included near to the point at which the vacuum line joins the receiver to exclude dust and micro-organisms when the vacuum is broken. Non absorbent cotton wool functions efficiently but may liberate tiny fibres, and needs frequent replacement. These disadvantages are less pronounced with nylon wool and absent when a small grade-3 sintered-glass filter is used.

The Matburn F 4 (Fig. 235) is a commercial unit that takes these points into account. It filters large volumes through a grade 3 or 4 sintered-glass disc in a pipeline holder and delivers the filtrate directly to the final container. The unit is attached by a clip to a spring-loaded tube mounted on a retort stand. It is pulled down towards the transfusion bottle and, when the rubber inside the glass hood contacts the rim of the bottle, the circuit is closed. Application of vacuum causes the unfiltered solution to flow from the container along a PVC tube through the filter and into the bottle via a glass delivery tube. The vacuum pump is connected, via a transfusion bottle that serves as a trap, to a glass jacket surrounding the delivery tube but open to the bottle at the bottom. On the trap is a control mounting by which the vacuum can be connected to, or cut off from, the jacket, when this is turned to the 'off' position, air filtered through a grade-3 glass filter is admitted and the unit springs off the bottle.

Simple units, suitable for filtering a single infusion fluid (a), single multi-dose injection and the solution for six ampoules (b or c) are shown in Fig 236.

After a sintered glass filter has been cleaned it should be well shaken, wrapped in smooth paper, labelled with the grade and dried in a warm oven. If it is left wet, pyrogens may be produced in the disc.

## FILLING

Although it is easier to keep the solution clean if filtration and filling are reduced to a single process, separation of the two stages is sometimes an advantage. Filling should be rapid, to save time and prevent recontamination with dust, but its speed is limited by the rate at which the measuring device (used for small volumes, transfusion bottles are usually filled to a graduation mark) can be filled, the ease with which liquid is able to flow out of this, and the time taken to change the container. The last of these factors depends on the dexterity of the operator (unless mechanical means are used) but flow into the final container can be facilitated by using a jet or needle of wide bore and, most important, filling of the measuring chamber can be accelerated by applying-

ing a higher vacuum or pressure. When similar pressures are used in a combined filtration-filling unit there is a risk of forcing particles through the filter, i.e. the filling rate in a combined process unit is controlled by the optimum speed for satisfactory filtration, there is no such limitation when filling is done separately. Pressure for filling a solution that oxidises readily should be provided from a nitrogen cylinder, not from a source of compressed air.

A very important feature of the design of filling equipment for ampoules is the method of obtaining accurate volumes, because the *British Pharmacopœia* specifies tolerances for the contents of this type of container.

## SEALING

Ideally, each container should be sealed immediately after filling.

With transfusion bottles, if the caps and plugs or liners are to hand there should be time to seal each while the next one is filling.

With multi-dose containers, the rubber plug or cap can be put on immediately and the rest of the closure fitted later. Bench machines are available for shaping the aluminium rings tightly around the necks of vials. For Clinbritic bottles the hand-closing tool (Fig. 23.12) is used, this is placed over the bottle neck and the two levers are pressed down to constrict a circular metal coil, this turns the lower edge of the ring under the bottom of the bottle flange. Excessive pressure must not be applied because heavy compression of the part of the rubber plug under the ring puts strain on the centre and causes it to bulge into the vial.

Separate filling and sealing of ampoules is often quicker and more efficient. Until filling is complete they should be collected in a clean metal or plastic tray or box under a small screen. For small numbers the most suitable sealing device is a twin jet burner (Fig. 23.7). The platform can be moved vertically to control the level at which the neck is sealed and, if the setting is not altered during the process, all the ampoules will be of the same height. In some sealers the position of the ampoule on the platform is fixed and the spacing of the burners can be adjusted (forwards and backwards, and laterally) for different

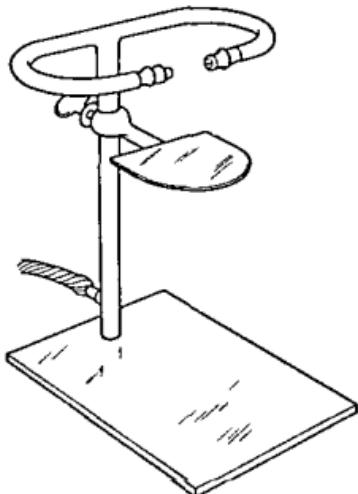


Fig. 23.7 TWIN JET BURNER  
(Courtesy Britton Malcolm & Co. Ltd.)

ampoule sizes. A large platform is preferable because it gives plenty of room for gripping the ampoule firmly so that it can be quickly removed at the optimum stage.

## INSPECTION

Injections must be examined for freedom from foreign particles, before and after sterilisation. The earlier inspection makes possible the refiltration of unsatisfactory solutions which may not be permissible

after sterilisation because of the harmful effects of a second sterilisation on certain medicaments

The particles may include fibres and matter of uncertain origin (in all injections), pieces of rubber (particularly in bottles) and fragments of glass (particularly in ampoules). As complete absence is very difficult to obtain and economically impractical, the standards accepted must be based on the highest possible clarity that experience has shown to be attainable with good filters, and unimpeachable technique. The tiniest fragment of glass or rubber is reason for rejection, as is the presence of even one large particle of any kind. The standard demanded for intravenous and intraspinal solutions must be particularly stringent.

The requirements for an inspection unit are critical illumination, protection of the observer's eyes from glare and a background painted half white and half black. The black is the most useful but the white allows rapid detection of dark particles such as char.

For ampoules and vials, a convenient arrangement is a box with a shielded lamp at the top. To reduce reflection it should be painted black inside, except for half the back. The container is held horizontally and rotated immediately under the lamp and then inverted once or twice to find heavy particles, such as glass. Movement must not be sufficiently vigorous to fill the solution with confusing air bubbles. Foreign particles in suspensions can sometimes be detected in the film left on the upper side as the container is slowly turned. Coloured containers are

difficult to inspect unless the colour is very pale.

A transfusion bottle is easier to examine when illuminated through the base. Most of the light rays become internally reflected and, therefore, the solution looks rather dark except for bright specks of light reflected from suspended particles. Contrast can be increased by blackening the inside of the examination chamber and by using baffles to keep as much room light as possible from the back wall (Norman, 1951).

A major difficulty is the dependence of the accepted standard on the personal opinion and eyesight of the inspector. Consequently, different inspectors, and even the same inspector at different times, may not examine uniformly. Graham *et al* (1959) studied some aspects of this problem and concluded that, in general, uniformity is achieved.

*With the aim of providing a method that would largely eliminate personal bias and give a numerical expression of the amount of suspended matter,* Hudson (1961) developed a very sensitive photoelectric nephelometer (an instrument that measures the density of a suspension from the amount of light reflected from it). The particle density in an injection is extremely small and, therefore, only a small amount of light is reflected, the current produced by this when it falls on a photocathode must be magnified many times before it can be detected on a microammeter. Hudson achieved this by using a photomultiplier, and was able to distinguish as little as 1 part per million of suspended solid.

(See also Godding, 1945.)

### TESTING AMPOULES FOR FAULTY SEALS AND CRACKS

Artz *et al* (1961) have listed four kinds of fault in ampoules—

1 A fine capillary through the centre of the seal. This is due to incomplete sealing and is more likely when the tip is just rotated in the flame than when the end is pulled off.

2 A crack at the tip, usually at the base of the seal. It is caused by strain from sealing too much glass into the tip, by jarring the ampoule while hot, or by rough treatment later.

3 A crack at the bottom of the ampoule, probably the result of careless handling during and after manufacture.

4 A hole at the tip, due to using too small a flame or removing the ampoule from it prematurely.

These workers compared several methods of detecting these faults and recommended—

(a) For heat sterilisable injections—immersion in a detergent-dye solution during or immediately after sterilisation, followed by washing and inspection for colour in the contents of the ampoules.

(b) For thermosensitive injections—immersion in the dye solution and application of a vacuum, return to atmospheric pressure forces the dye into faulty ampoules.

Complete immersion is necessary, if only the tips are covered, cracks in the bases may escape detection. The dye must be harmless, in case its entry is not detected, and, therefore, a permitted food colour (Ministry of Food 1954 1955) such as amaranth, or one of the substances used for medical diagnostic procedures, such as methylene blue or rose bengal, must be chosen. Bordeaux B, once widely used as a pharmaceutical colouring agent, is an example of a non permitted dye.

For testing during autoclaving the ampoules are immersed in a suitable container such as a stainless-steel tray, drum or beaker, taking precautions, e.g. prepacking in a weighted muslin bag, to keep them under the surface. Enough time must be allowed for penetration of heat to the centre of these vessels because, in effect, a large number of easily heated small containers have been replaced by one relatively large one that will heat slowly. For this reason a flat tray is preferable because rapid penetration will take place in one direction. Post sterilisation testing has the advantage of not prolonging the heating up but, to ensure that a significant amount of the dye is taken in, the ampoules must be transferred to the solution before they have cooled too far. A large vacuum dessicator is suitable for the test on thermolabile injections.

Ampoules with coloured contents, or of coloured glass, cannot be tested with confidence by dye

methods but Artz *et al.* mention another technique that would be applicable. In a special apparatus, a powerful air current is blown on the tip and, separately, the base, the appearance of bubbles in the ampoule contents indicates a fault. Another method for coloured solutions is to cover each ampoule with water in a separate test tube, sterilise the tubes upright, and reject those in which the water becomes coloured. The seals of a small batch of coloured ampoules can be checked by shaking the contents into the neck and cooling the body in a stream of water, as the contents contract, air bubbles, sucked in through the faults, can be seen rising through the solution.

Grossly unsatisfactory sealing can be detected before sterilisation by the droplets that escape when the inverted ampoules are shaken over the palm of the hand.

## OVERSEALS

These are necessary on transfusion and multidose containers to show that no-one has tampered with the closures or contents. They are put on after sterilisation.

### TRANSFUSION BOTTLES

For a complete cap with a flat liner, a plastic ring round the cap-bottle junction is adequate but when, as is much more common, the closure is a plug with a centrally perforated cap, an overseal that covers the top is more suitable because it protects the exposed rubber. The seals dry more quickly if applied while the bottles are still warm from the autoclave.

Plastic overseals must be stored in a preservative fluid. They take up bench space, and they sometimes split in use. Because of these disadvantages, aluminium foil overseals have been recommended as alternatives. They can be used and stored dry, are durable, may be embossed with a warning against use of the contents if the seal is broken, and can be applied quickly with a special sealing machine.

For certain injections, industry uses a type of tamper proof seal that, if cost allowed, would be excellent for transfusion bottles. A metal cap is spun on to the bottle so that it fits the threads accurately, but the part below the lowest thread is connected to the rest at a few points only (Fig. 23.8). When the

cap is unscrewed the connections break and the ring of metal falls to the neck of the bottle to give a clear indication that the container has been opened.

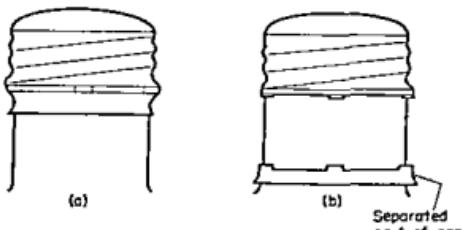


Fig. 23.8 TAMPER PROOF SEAL  
(a) Before and (b) after opening

### MARK 1 CLINBRITIC BOTTLE

The use of plastic rings for this bottle is explained in the chapter on containers. After sterilisation, sufficient time must be allowed for the bottles to cool because if the bakelite cap is removed too soon the high internal pressure will blow out the rubber cap and thus make the injection unusable. The ring should be wide enough to cover the cap-bottle junction and leave sufficient to turn slightly over the top of the cap.

## PACKAGING

### TRANSFUSION BOTTLES

In hospitals these are usually issued unwrapped because of the time and expense involved in wrapping considerable numbers of large bottles. Therefore, storage after issue should be in a dust-proof cupboard, and protection from dust during transport is necessary. When wrapping is used transparent films are most suitable because they keep clean longer than white demy and do not hide the labels.

### MULTIPLE DOSE CONTAINERS

The usual alternatives are transparent film or a box. The latter is more costly and needs separate labelling but, after use, the bottle can be returned to it for protection from dust and, if necessary, light. Amber wrapping film is available for light protection but is unlikely to survive the initial opening, after which storage in a dark cupboard is required.

### AMPOULES

Large ampoules are packed singly but others are issued in boxes of five or six. Boxes should be stout enough to withstand normal risks of handling and transport and must be subdivided to keep the contents from knocking against each other.

'Grip-tight' boxes are most popular today. In these the base of a hinged box contains smooth, white corrugated card, and each corrugation holds

one ampoule. Even if the box is inverted with the lid open the contents will not fall out.

Boxes in transparent polystyrene are a recent development, the ampoules being gripped in compartments of a more flexible plastic. Their bright and clean appearance makes them most acceptable for a sterile product. For light-sensitive substances, amber boxes are desirable.

Gillanders (1956) introduced a robust, enamelled steel box to replace short-lived cardboard containers for the issue of ampoules to wards.

A small ampoule file must be included unless ampoules that do not require filing have been used.

The box is most suitably wrapped in transparent cellulose film. Two main types are in common use, one is sealed by very slightly damping the edges with water or an adhesive, the other requires heat and pressure which are applied with a temperature-controlled sealing iron.

In all cases, containers must be completely dried and carefully polished before packing. To avoid cloths, a wash in warm non-ionic detergent (non-ionic types do not leave a deposit on drying) followed by rinsing in distilled water and drying in a warm atmosphere can be used. Although they should not leak it is safer not to immerse the closures of bottles during washing. Precautions are taken to avoid finger-marks on ampoule necks during boxing.

## FLOW PRODUCTION OF PARENTERAL SOLUTIONS

The production of sterile fluids involves so many processes that full efficiency can be obtained only by careful work study. Laboratories, benches and equipment must be arranged to produce a direct flow from one stage to the next and to provide, conven-

tiently at hand, everything needed by an operator working on a particular procedure. It is particularly important to keep clean areas as far from dirty ones as possible.

## EXAMPLES

The following selection includes the different types of injection and methods of sterilisation. The vol-

umes and batch sizes are representative of those commonly made in course work in schools of pharmacy.

### EXAMPLE 23.1

#### *Sterilisation method*

#### *Type of injection*

#### *Example*

Heating in an autoclave

Single dose of small volume

Six ampoules of Histamine Acid Phosphate Injection B.P.

1. The B.P. states that when no strength is prescribed, as in this case, a solution containing 1 mg in 1 ml is to be dispensed.

(continued overleaf)

*Example 23 I continued*

2 Two alternative methods of sterilisation are given—Heating in an autoclave, or filtration. The former would be chosen because heating processes are more reliable. The reason for allowing filtration as an alternative is discussed under 'Sterilisation by Filtration'.

No bactericide is required, either as an aid to sterilisation (because autoclaving is being used) or as a preservative (because the injection is single dose). Therefore, the preparation of the solution is very simple and merely involves dissolving the medicament in Water for Injection or, since the solution will be sterilised immediately after preparation, freshly-distilled pyrogenic water.

3 The container must comply with the limit test for alkalinity of glass because histamine acid phosphate is an acid salt.

4 *Overage*

When a single-dose injection is dispensed in an ampoule a slight excess must be included to cover the volume that cannot be removed from the ampoule during withdrawal of the dose, the drop that is often lost when air is displaced from the needle just before injection, and the liquid that is left on the inner surface of the syringe and within the needle after administration. If these losses were ignored the patient would not receive the full dose.

The excess is often called overage, and the amounts for different sizes of ampoule are given in a table in the B.P. Separate values are listed for viscous (oily) and mobile injections, for the former they are larger, chiefly because more will be left on the walls of the ampoule. For mobile preparations, overages range from 20 per cent for a 0.5-ml ampoule to 2 per cent for sizes greater than 20 ml.

As the B.P. also gives volume tolerances, filling must be performed very carefully. An apparatus suitable for very accurately checking the volumes of injections in ampoules, for control purposes, has been described by Scott (1952).

5 *Formula*

For a 1 ml ampoule the overage is 0.1 ml and, therefore, for 6 a total volume of 6.6 ml of solution is required. To allow for manipulation losses and for the preparation of additional ampoules in case leaks or particles are found in any of the first six, 10 ml should be prepared. With bigger ampoules it is unnecessary and very wasteful to prepare, as in this case, ten times the prescribed dose when only six ampoules are required. For example, for six 10-ml ampoules of Calcium Gluconate Injection the volume to be dispensed, with overage, is  $6 \times 10.5 = 63$  ml, and 70 ml (seven times the prescribed dose) would be an adequate working quantity. It is especially important to make the working excess as small as possible when the medicament is expensive (e.g. emetine hydrochloride) or a dangerous drug. In the latter case any residual ampoules or solution must be retained in the dangerous drug cupboard.

Taking the above points into account the formula becomes—

	B.P. Amounts	Amounts used
Histamine acid phosphate	1 mg	10 mg
Water for Injection	to 1 ml	to 10 ml

6 *Calculation*

10 mg is not a weighable quantity. Therefore, 50 mg is weighed, dissolved in a suitable volume of solvent and finally made up to 50 ml.

*7 Labels*

As histamine acid phosphate is not a poison the labels are comparatively simple. If colourless ampoules are used a warning of the need for protection from light must be given on the box.

*Ampoule*

HISTAMINE ACID
PHOSPHATE
INJECTION B.P.
1 mg in 1 ml

*Box*

PROTECT FROM LIGHT
--------------------

Six Ampoules
HISTAMINE ACID PHOSPHATE
INJECTION B.P.
1 mg in 1 ml
Sterilised 5.9.63 (1) Name

Although one label of each type must be written before preparation of the solution is begun, it is not essential to write all the *ampoule* labels at this stage. Time can be saved by leaving the rest until the injection is being sterilised.

*8 Preparation of the Ampoules*

(a) *Opening*. Ampoules as received from the manufacturer are usually sealed to exclude dust. A small file mark is made on the neck at a suitable place. Several filing devices are available, including the simple strip of metal with a serrated edge that is commonly enclosed in boxes of finished ampoules, a triangular, fine grade carborundum stick, 4 in. x  $\frac{1}{2}$  in., obtainable from cutlers, and various diamond tipped cutters. The position of the mark depends largely on the length of the filling needle, the tip of this must enter the ampoule body because deposition of solution in the neck may cause an air lock and overflow, and charring may occur during sealing. Also, removal of the needle without touching the neck is more difficult if the latter is long. Nevertheless it must not be too short because it widens towards the base and, therefore, becomes harder to seal. A further point to watch, particularly during sealing, is that the ampoules are short enough to fit into their box.

A simple piece of equipment, the Manning ampoule cutter, consists of a frame carrying a mounted diamond and an adjustable gauge plate, on which the ampoule base rests for fixing the length at which the neck is to be filed (Fig. 239). The neck is drawn across the diamond and at the same time, the ampoule is given a reverse rotational movement, this produces a

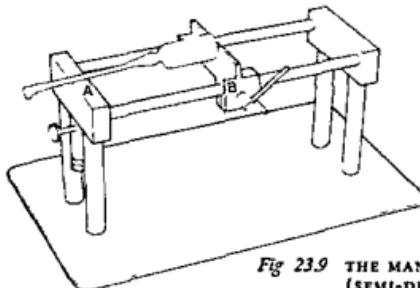
(continued overleaf)

*Example 23 I continued*

short scratch line. Using this apparatus, a batch of ampoules can be rapidly filed at the same length.

In filing by hand the ampoule is held with the neck pressed firmly against and supported by, the front of the left forefinger.

Each ampoule is opened by holding it inverted in the left hand and snapping off the end of the neck with the right by pulling sharply and gently pressing away from the file mark. To minimise splintering, pulling should predominate. The ampoule is shaken sharply before reverting. This method of opening reduces the number of splinters, encourages them to fall away from the ampoule mouth and removes most of the pieces that have entered the neck.



*Fig. 23.9 THE MANNING AMPOULE CUTTER  
(SEMI-DIAGRAMMATIC)*

*A—diamond B—adjustable gauge*

A further method of opening is to use a hot-wire cutter. In this the ampoule neck is rolled on an electrically heated wire and the ends are gently pulled apart. It gives a cleaner cut.

(b) *Annealing*. The mouth of each ampoule is rotated in a Bunsen flame to melt down the rough edge. If this is not done glass fragments may be found in the finished ampoules in spite of carefully washing before filling. These fragments have been rubbed off the rough tip by the point of the filling needle or, less often, through allowing the forceps to slip across the mouth during sealing.

(c) *Washing*. Ampoules are supplied clean but washing before use is essential to remove glass fragments from opening and traces of dust from manufacture.

If a special washing unit is not available a syringe can be used. The ampoule is held inverted over a sink and a syringeful of freshly-distilled apyrogenic water is forcibly injected into it, making sure that the jet hits the base. The residual water is shaken out and the process repeated. Then the ampoule is inverted in a small beaker to drain.

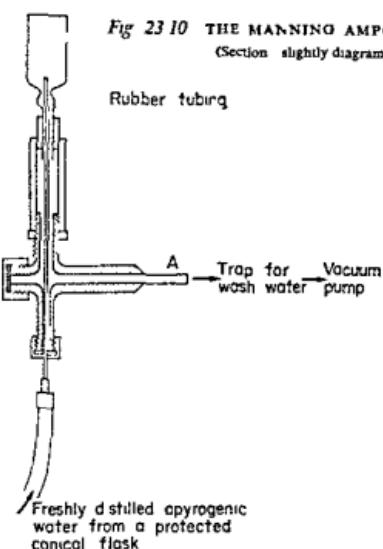
A simple commercial unit is shown in Fig. 23.10. The flask is filled with water (the use of this word in italics should be interpreted as freshly-distilled apyrogenic water in this and the following chapters). The ampoule is inverted over the needle and pressed down so that its neck makes a good seal with the rubber tubing. Vacuum causes a stream of water from the needle to wash the ampoule thoroughly and quickly.

For other examples of washing devices, see Blenkiron (1953), Boehm (1955), Davies and Millar (1948) and Wood (1952).

(d) *Drying*. The inverted ampoules are dried, in the beaker, in a drying oven. If they were used wet the strength of the injection could be

significantly lowered. Also, when a wet ampoule is filled the medicament often diffuses quickly up to the neck through the moisture film on the inside and then charring occurs on sealing. This is particularly noticeable when soluble sulphonamides are put into wet ampoules.

*Fig. 2310 THE MANNING AMPOULE WASHER  
(Section slightly diagrammatic)*



On a large scale, each ampoule can be inverted in a hole large enough to take its neck, in a metal platform that, after drying, can be transferred to a lidded metal box for clean storage.

The cooled ampoules are placed upright on the bench or in a suitable stand. Each is covered with a small glass tube (e.g. a Durham tube) to protect its mouth from dust. Ampoules are easily knocked over if a stand is not used and one can be made simply by boring holes of suitable size in a piece of hard wood.

#### *9 Preparation of the Solution*

Measure 50 ml of water from a transfusion bottle (a suitable container for the solvent) into a cylindrical measure.

Transfer 20 ml of this to a 100-ml conical flask and cover the measure and flask suitably.

Weigh 50 mg of the medicament on a chemical balance and transfer it to the flask, using more water from the measure to rinse the scale pan. A small funnel is helpful when the weight is small or the solubility high.

Dissolve, using gentle heat, occasionally swirling the contents of the flask.

Add most of the rest of the water from the measure.

*(continued overleaf)*

*Example 23 I continued*

### 10 Filtration

Gravity filtration through a grade-3 sintered glass filter is satisfactory because the volume is small

A good receiver for the filtrate is a conical measure. From this it is easy to withdraw the solution with a syringe (compare a small conical flask) and, particularly, to remove the last few doses (compare a beaker, which has to be tilted)

The filter funnel is supported in a stand and protected from dust during filtration. The measure is covered with cellulose film in which a hole has been cut for the funnel stem. This hole should not be made by forcing the stem through the paper. It is not uncommon to find a small piece of film, torn off by this procedure, in a multi-dose container that has been filled directly from the filter.

If the filter has been properly prepared it will be dry and the injection can be made up to volume before filtration. If, however, it is thought desirable to rinse the filter with solvent before use the volume should be adjusted through the filter after filtration, to prevent dilution of the solution by water retained in the interstices of the disc.

### 11 Filling

Six ampoules are most conveniently filled with a syringe.

The main aims must be to measure the volumes accurately and to keep the neck free from liquid. Many medicaments are organic chemicals that char on heating and if there is solution at the point of sealing, an ugly black ring is produced from which pieces may separate and contaminate the injection during sterilisation and storage. The following method of filling is practically foolproof—

(a) Carefully draw into the syringe a little more than 1 1 ml of the solution. Do this gently to avoid large numbers of air bubbles in the liquid.

(b) Invert the syringe to allow the air to rise towards the needle and push up the plunger to expel the bubble and leave exactly 1 1 ml in the barrel.

(c) Wipe the needle with a cellulose film disc, unlike filter paper or a cloth, this will not leave fibres on the needle.

(d) Invert the ampoule over the needle. Since the surface of the latter is dry, no liquid will be put on the ampoule neck. If, however, the ampoule is held upright and the syringe is reverted for filling, the weight of the plunger often forces a drop of solution from the tip of the needle, and contamination of the neck is hard to avoid.

(e) Once the ampoule is safely over the needle, which must be long enough to reach well into the ampoule body, revert both container and syringe together and expel the liquid, gently and slowly to prevent splashing into the neck.

(f) Finally, touch the needle tip against the constriction at the bottom of the neck to dislodge the last drop of liquid and then sharply withdraw the needle without touching the neck.

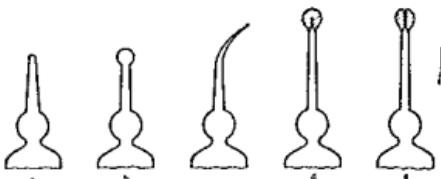
During filling each Durham tube can be held in the little finger of the right hand and immediately replaced afterwards.

### 12 Sealing

Use a twin jet burner. Set the platform at a satisfactory height and adjust the flames to a suitable intensity.

The composition of ampoule glass varies and some batches melt more easily than others. Check the behaviour of the type being used by trying an empty, but open ampoule before sealing the batch.

Remove the Durham tube. With the left hand position the ampoule between the two flames. Grip the end of the neck with blunt nosed forceps held in the right hand and when the glass is soft enough pull off the top vertically and gently. Leave the tip in the flame a second or two longer and then remove. The tip should be smoothly and evenly rounded.



*Fig. 23 II GOOD AND BAD AMPOULE SEALS*

with a small flat blob of glass in the centre (Fig. 23 IIa). The two most common faults are—

- (a) Leaving the tip in the flame too long after sealing when expansion of the air inside causes the glass to balloon (Fig. 23 IIb)
- (b) Pulling off the top too vigorously, this draws the tip into a fine sharp point that is easily broken and can give a painful prick when the ampoule is handled (Fig. 23 IIc)

If a twin jet burner is not available sealing can be done in a Bunsen flame. The ampoule is rotated almost horizontally with about  $\frac{1}{8}$  in. of the neck in the non-luminous flame. When the glass starts to bend the tip is very slowly withdrawn with appropriate rotation to correct the bending and completely removed when there is no longer a continuous hair line where the glass has annealed. The resulting seal is elegant and very strong but requires considerable practice to perfect (Fig. 23 IID). The commonest fault is to leave a narrow capillary (Fig. 23 IIE) this can often be detected by a small depression at the tip. To ensure sealed ampoules of equal size the necks must be cut to the same length since no part is removed unlike the twin jet method.

### 13 Preliminary Inspection

Test for grossly unsatisfactory seals by shaking and examine for particles. If necessary prepare extra ampoules to replace rejects.

### 14 Sterilisation

If the seals are to be tested during sterilisation use the minimum volume of dye solution to cover the ampoules because the heating up time will depend on the volume in the tray or beaker. Colour the water deeply. Solution of Amaranth B.P. is probably the most convenient preparation for this purpose.

If several batches of the same size of ampoule are being sterilised at the same time include means of identification.

*(continued overleaf)*

*Example 23.1 continued*

Carry out the sterilisation procedure appropriate to the type of autoclave being used. When it has reached pressure or temperature remember to record the times of the beginning and end of the exposure.

After removal allow the beaker to cool sufficiently for dye to be drawn into faulty ampoules as their contents contract.

If seal testing is carried out after autoclaving, remove the ampoules to a beaker of warm (cold might cause breakages) dye solution and leave for a few minutes. Transfer from the autoclave to the dye solution is facilitated if the ampoules are sterilised in a muslin bag. The latter is weighted (e.g. with glass beads), to ensure that the ampoules are fully immersed in the dye, and tied with twine, one end of which is left long for holding.

Examine and reject any coloured ampoules.

*15 Final Inspection*

Rinse in a non ionic detergent solution and then in distilled water, to remove dye adsorbed on to the glass. This is easier if post sterilisation dye immersion is used. Again inspect for freedom from particles and, if necessary, discard any unsatisfactory containers.

*16 Labelling and Packing*

Carefully check the labels and stick them on carefully. Then polish the ampoules, taking particular care to remove gum left from altering the position of a label. Put the ampoules into the box without finger-marking their necks, include an ampoule file and wrap in cellulose film.

**EXAMPLE 23.2**

*Sterilisation method* Heating in an autoclave

*Type of injection* Single dose of large volume

*Example* 500 ml of Dextrose Injection B.P.

1 The B.P. directs a 5 per cent w/v solution when no strength is prescribed.

2 Autoclaving is more appropriate than filtration.

Bactericides are not allowed in this type of injection.

Overage is unnecessary.

Therefore, the preparation involves only solution of the medicament in the solvent.

*3 Formula*

No excess is made because there are no manipulation losses.

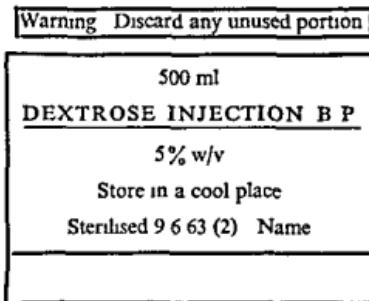
	<i>B.P. Amounts</i>	<i>Amounts used</i>
Dextrose	5 G	25 G
Water for Injection	to 100 ml	to 500 ml

*4 Labels*

Two special labels are needed—

(a) A warning against the use of the contents on more than one occasion. This is desirable on all infusion fluids.

## (b) A direction to store in a cool place

*5 Preparation of the Solution*

There are two forms of dextrose in the Pharmacopæria—Dextrose (Anhydrous dextrose) and Dextrose Monohydrate—and care must be taken to use the right one. The monohydrate is not sufficiently pure for the preparation of injections and on autoclaving its solutions become brown due to caramelisation.

Transfer about 250 ml of solvent from a full 500-ml bottle to a protected 500-ml conical flask, replace the bottle cap.

Weigh the dextrose, transfer it to the flask and gently warm, with occasional shaking, until solution is complete. Make up to about 400 ml. With dextrose it is particularly important not to add the solvent to the medicament because a slowly soluble sticky mass can result. Also, if the powder is allowed to adhere to the bottom of the flask during heating, charring will occur.

*6 Filtration*

This volume requires vacuum assisted filtration and, therefore, a grade 4 filter can be used. The apparatus shown in Fig. 23 6a would be suitable with a 1-litre flask carefully calibrated at 500 ml.

Adjust the vacuum so that the solution comes through at no more than a fast drip, critically examine the filtrate for particles and, if necessary, refilter. Sometimes, several filtrations are required because freedom from foreign particles is so very important for intravenous fluids.

Make up to volume through the filter after using the adjusting solvent to rinse the preparation flask.

*7 Filling*

A 500-ml transfusion bottle is a suitable container. Assuming that it has been stored with a double paper cap protecting the mouth, discard the outer one, carefully remove the other, check that the bottle neck is not chipped, pour in the solution and immediately replace the inner cap.

If a plug type closure is to be used, take one from its storage vessel with forceps. Lift the paper cap from the bottle again and push the plug into the neck, taking care not to touch the part that goes into the bottle. Take a ring type metal cap from its storage tin, inspect it for freedom from deformities and then screw it on the bottle tightly. Fix a new paper cover over the cap with an elastic band, this is to prevent aerial contamination of

(continued overleaf)

*Example 23.2 continued*

the exposed rubber after the bottle has been removed from the steriliser and until the plastic cap has been fitted

When the alternative closure is used, remove a flat liner from its storage container with forceps, place it over the mouth of the appropriate cap and press it into position, preferably with a thumb covered with a clean rubber thumb-stall. Remove the paper cap from the bottle and screw on the closure tightly.

If the plug or liner has been stored in sterile water it should be rinsed in several changes of solvent before use because when rubber closures are kept in this way particles sometimes separate, these may cling to the closure as it is removed and later contaminate the injection, if not rinsed off.

Invert and shake the bottle to confirm that the seal is satisfactory. Then examine the solution critically for particles and refilter if necessary, it is advisable to use a new bottle and closure after filtration.

**8 Sterilisation**

The most important feature is the extra time required for heating the volume to sterilisation temperature. Usually this will be known from a previous experiment. Assuming, for example, that it is 15 min, a total holding time of 30 (exposure) + 15 (lag) = 45 min is necessary.

After sterilisation, and when the pressure has fallen to zero, open the vent, loosen the lid slightly and leave the bottle inside for at least 10 minutes longer to reduce the chance of breakage when it is removed to the cool air outside.

**9 Sealing**

Put on a plastic seal as soon as possible.

**10 Polishing and Examination**

Polish the bottle when it is cool. It may be necessary to dip it into non ionic detergent first to remove stubborn splash or drip marks from the autoclave. Re-examine for particles and reject if necessary.

**11 Labelling**

Check and stick on the labels. Place both near to the top front of the bottle to ensure that the warning label is not overlooked. If the rubber plug is to be used twice, a label must state which quadrants are appropriate.

**12 Issue**

Injections must never be issued hot, but remember that cooling under a tap is dangerous and often leads to breakage.

**EXAMPLE 23.3**

*Sterilisation method* Heating in an autoclave

*Type of injection* Multiple dose

*Example* 30 ml of Stibophen Injection B.P.

1 The B.P. gives a definite formula for this injection, i.e. unlike the previous examples, only one strength is recognised.

2 Since 30 ml is prescribed and the maximum dose is 5 ml, this is a multiple dose injection and, therefore, a bactericide, in preservative concentration, must be included. No incompatibilities have been reported between stibophen and any of the four officially recommended bactericides but either chlorocresol or phenylmercuric nitrate would be first choice.

because, usually stock solutions are kept in a sterile products department where they are also needed for Heating with Bactericide. The strengths normally provided are 0.2% w/v chlorocresol and 0.002% phenylmercuric nitrate. Apart from the convenience of having on hand ready-made solutions of these frequently-used substances, there are other reasons for the practice, the small amounts that are often required in injections necessitate awkward triturations and, probably most important, both compounds are only slowly soluble. In addition, chlorocresol is volatile in steam and attempts to dissolve it quickly by boiling vigorously in an open vessel can cause considerable loss into the atmosphere. This explains the claims, sometimes made by students, to have dissolved this substance considerably in excess of its solubility, which is 1 in 260 at 20°C, i.e. less than 0.4 per cent. A satisfactory way of preparing stock chlorocresol solution is to put the required amount with almost the total volume of water in a stoppered graduated flask and leave it in a warm place, with occasional shaking, until solution is complete. Then the volume is finally adjusted. Suitable containers are transfusion and McCartney bottles, the rubber liners of which have been equilibrated with the appropriate bactericide.

3 The container must comply with the limit for alkalinity of glass. The optimum pH range for stability is 5.0 to 5.5 and a buffer is added to help to maintain this. A Clinbritic bottle is suitable. If it is assumed that 5 ml is the most likely dose the container will contain only five full doses, allowing for losses in the syringe, i.e. the requirement that a multi-dose container should not contain a large number of doses is complied with.

#### 4 Overage

It is not customary to supply overage with a multiple dose injection. This is chiefly because this method of packing is intended to have the advantage of flexibility of dose volume. For example, the dose for Stibophen Injection may range from 1.5 to 5 ml and if ampoules were used to cover this, a series of different sizes would be needed to avoid wastage. In these circumstances it is not possible to decide exactly how much overage is necessary and, consequently, although the contained volume is given on the label, no claim is made that the container holds a definite number of doses.

#### 5 Formula

Chlorocresol (0.1%) will be used as the bactericide because it is less readily lost from rubber-sealed containers than phenylmercuric nitrate.

	B P Amounts	Amounts used
Stibophen	6.4 G	1.92 G
Sodium acid phosphate	0.25 G	0.075 G
Chlorocresol solution 0.2%	50 ml	15 ml
Water for Injection	to 100 ml	to 30 ml

#### 6 Calculation

At first sight it would appear that the way to prepare this solution is to dilute sufficient stock 0.2 per cent chlorocresol solution with an equal volume of water and use the dilution as the solvent. However, to obtain exactly 0.1 per cent of chlorocresol in the injection exactly 30 ml of the dilution is necessary, but this is not possible because of the displacement caused by the two ingredients. Usually, the resulting error is so small that

(continued overleaf)

*Example 23.3 continued*

it can be accepted (as was done on p. 355) but it is preferable to adopt the following more accurate method.

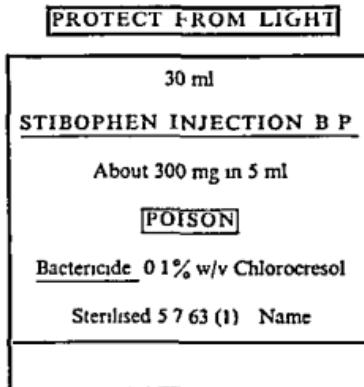
Dissolve the ingredients in less than 15 ml of water and make up to 15 ml with the same solvent. Then add an equal volume of 0.2 per cent chlorocresol solution. The solution will then contain the exact amount of bactericide.

Although the weight of each ingredient is more than 50 mg the weighing of the last figure of the 75 mg of sodium acid phosphate may present difficulty if the balance has no rider. Then, 100 mg can be weighed, dissolved in water to 12 ml, and 9 ml ( $\equiv$  75 mg) used.

**7 Labels**

The assumption will be made that supply is under conditions where full poisons law labelling is necessary. Stibophen Injection is a Schedule 1 poison and must be labelled with 'Poison' in red.

If a clear glass bottle is used, a label instructing protection from light is required.



Guidance on the way to express the strength can often be obtained, as in this case, from a statement at the end of the B.P. monograph.

**8 Preparation of the Solution**

Using bulb pipettes and water, accurately calibrate a hard-glass boiling tube at 15 ml and 30 ml. Leave about 10 ml of water inside.

Weigh 100 mg of sodium acid phosphate and dissolve it in the water using gentle heat. Cool and make up to 15 ml.

Remove 3 ml with a bulb pipette. The remaining 9 ml contains the required amount of buffering agent.

Weigh the stibophen, have it checked and dissolve it, using slight heat if necessary, in the buffer solution. Cool and make up to 15 ml with water.

Finally, add 0.2% chlorocresol to 30 ml. Stir thoroughly to mix.

The tube, provided it is rimless, can be protected with a clean loose-fitting aluminium test tube cap. Often this method of protection is also applicable to small conical flasks.

As much use as possible should be made of bulb pipettes for accurate measuring of small volumes in the preparation of parenteral solutions.

### 9 Filtration

Gravity filtration, as in example 23 1, would be suitable, but as the volume is three times as large in this case it is quicker to use a grade-4 filter with vacuum. The apparatus shown in Fig 23 6b is satisfactory and if permits filtration directly into the final container.

Push a pad of plastic foam to the bottom of the receiver, this helps to steady the bottle and raises it to a suitable height. Put in the container and tightly insert the bung, taking care that the stem of the funnel is inside the bottle neck. Fix the apparatus to a clamp stand and connect to vacuum. Filter at a slow drip. As the solution was made up to volume before filtration, the filter must be dry.

Examine for particles and, if necessary, refilter into a new bottle. The original container is not re-used because it would have to be washed to remove adherent particles and the residual wetness might dilute the solution significantly.

Immediately after removal from the unit, loosely cover the mouth of the bottle with its bakelite cap.

### 10 Capping

(a) *Using a Skirted Cap*. With forceps remove a cap from the storage solution in which it has been equilibrating. Rinse it in several changes of

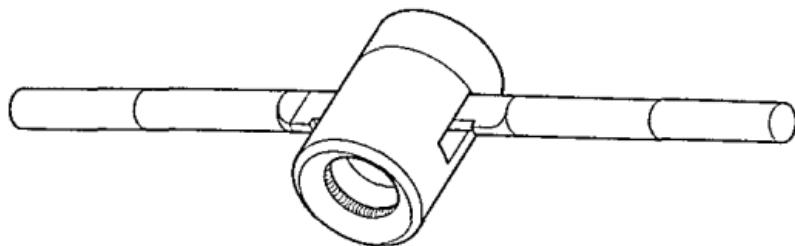


Fig 23 12 CLOSING TOOL.

the vehicle (in this case 0·1% chlorocresol solution) to remove any adherent particles, shake off excess liquid and put the cap on the bottle without touching with the fingers the part that goes into the neck. Screw on the bakelite cap tightly.

(b) *Using a Plug and an Aluminium Ring*. Insert a plug, obtained from the storage solution and rinsed as above, place an aluminium ring over it and crimp this into position with the closing tool (Fig 23 12) without using too much pressure. Screw the bakelite cap on tightly.

### 11 Sterilisation

There are no special features.

### 12 Sealing

If a skirted cap was used fit a plastic ring to the neck of the bottle, when the latter has cooled sufficiently. There is unlikely to be enough heat left in the container to harden the ring quickly and, therefore, drying should be accelerated, e.g. in a slightly warm oven.

(continued overleaf)

*Example 23.3 continued*

For the reasons given in the chapter on containers, no antiseptic should be put on the porous pad in the bakelite cap

**13 Packing**

After polishing, inspection and labelling, either wrap the bottle in amber cellulose film or pack in a fully labelled white cardboard box.

The *United States Pharmacopoeia* requires a multi-dose container in an opaque carton or wrapper to carry a statement that the covering is required for protection from light until the contents have been used. A similar statement should be put on the box or film used for this preparation so that the covering is not thrown away at the first time of use

**EXAMPLE 23.4**

<i>Sterilisation method</i>	Heating with a Bactericide
<i>Type of injection</i>	Single dose of small volume
<i>Example</i>	Six 1-ml ampoules of Morphine Sulphate Injection each containing 10 mg of morphine sulphate

1 Notice that the number of ampoules and the amount in each have been stated, in accordance with Dangerous Drug Regulations

2 Heating with a Bactericide and filtration are the alternative methods of sterilisation but for the reason stated in Example 23.1 the former will be used

3 The ampoules must comply with the limit test for alkalinity

**4 Overage**

0.1 ml/ampoule

**5 Formula**

Two inclusions are necessary—

(a) The bactericide—morphine sulphate is compatible with chlorocresol or phenylmercuric nitrate, the former will be used, for the reason given in Example 23.3

(b) An antioxidant—0.1 per cent sodium metabisulphite is directed in the monograph.

	<i>Amounts prescribed</i>	<i>Amounts used</i>
Morphine sulphate	10 mg	100 mg
Sodium metabisulphite	1 mg ( $\equiv 0.1\%$ )	10 mg
0.2% chlorocresol solution	to	1 ml to 10 ml

**6 Calculation**

0.1% sodium metabisulphite	$0.1\% \equiv 0.1\text{ G in }100\text{ ml of solution}$ $\text{or }100\text{ mg in }100\text{ ml of solution}$ , $\text{i.e. }1\text{ mg in }1\text{ ml of solution}$ .
----------------------------	--

Trituration for 10 mg—

Sodium metabisulphite	50 mg
0.2% chlorocresol solution to	25 ml
	$1\text{ e }2\text{ mg/ml}$ Take 5 ml.

The morphine sulphate is dissolved in this 5 ml and the solution made up to 10 ml with more solvent.

Notice that the slight reduction in the final concentration of chlorocresol caused by using a stock solution has been ignored in this case. The alternatives are—

(a) To use solid chlorocresol—objections to this were given in Example 23 3

(b) To use a more concentrated solution and follow the method of Example 23 3. Although an 0·004% solution of phenylmercuric nitrate can be prepared 0·4% is beyond the solubility of chlorocresol at room temperature. 0·3% could be used but the calculation and preparation would be more involved and might lead to errors far more serious than the displacement error.

#### 7 Labels

Assuming the same conditions of supply as in the previous exercise account must be taken of the fact that morphine sulphate is a Dangerous Drug. Examples of the labels required are given in the section on labelling but in this case the strength is different.

#### 8 Preparation of the Solution

In a hard glass boiling tube dissolve 50 mg of sodium metabisulphite in about 10 ml of 0·2% chlorocresol solution using gentle heat. The solution must not be boiled because of the volatility of the chlorocresol in steam. Transfer to a measure and make up to 25 ml with solvent rinsings of the tube.

With a bulb pipette remove 5 ml to another tube and dissolve the weighed and checked morphine sulphate in this.

Transfer to a 10 ml measure and make almost up to volume with rinsings from the tube. Final adjustment can be made now or through the filter.

All other stages are as for Example 23 1, except—

#### 9 Sterilisation

About  $\frac{3}{4}$  fill a large (e.g. 600 ml beaker) with water and put the ampoules inside. To ensure complete immersion and for convenience of removal it is useful to enclose them in a weighted muslin bag with a string attached that can lead out over the lip and be taped to the outside of the beaker. To prevent damage from bumping a pad of absorbent cotton wool can be placed at the bottom of the beaker.

Cover the mouth of the beaker with a clock glass.

Raise the water to boiling and keep it there throughout the exposure period.

#### 10 Testing the Seals

Immediately after sterilisation add sufficient dye to colour the water deeply and leave to cool. If steaming is used instead of heating in a beaker of water the post sterilisation method of seal testing described in Example 23 1 is most suitable because it avoids immersion in a beaker during sterilisation and the consequent lengthening of heating up.

#### 11 Retention of Excess

As this is a Dangerous Drug spare and rejected ampoules and any excess should be kept in the Dangerous Drug cupboard.

**EXAMPLE 23.5**

*Sterilisation method* Heating with a Bactericide  
*Type of injection* Multiple dose  
*Example* 20 ml of Procaine and Adrenaline Injection B.P.

- 1 The complete formula is given in the Pharmacopoeia,
- 2 This injection may only be sterilised by Heating with a Bactericide
- 3 The container must comply with the limit test for alkalinity

**4 Formula**

This contains only 0.1% chlorocresol but, as explained under Heating with a Bactericide, it gives, together with the other ingredients, a solution of adequate bactericidal activity. No additional bactericide is required.

	B.P. Amounts	Amounts used ( $\frac{1}{5}$ B.P.)
Procaine hydrochloride	2 G	0.4 G
Sodium chloride	0.5 G	0.1 G
Chlorocresol	0.1 G	0.02 G
Adrenaline solution	2 ml	0.4 ml
Sodium metabisulphite	0.1 G	0.02 ml
Water for Injection	to 100 ml	20 ml

**5 Calculations**

The easiest way of providing the chlorocresol is by using 10 ml of an 0.2% solution ( $= 0.02 \text{ G}$ )

The only other unweighable quantity is the amount of sodium metabisulphite which is obtained as follows—

Sodium metabisulphite	50 mg
Water	to 20 ml (i.e. 10 mg/4 ml)
Take 8 ml ( $\equiv 20 \text{ mg}$ )	

This is added to the chlorocresol solution.

The adrenaline solution is added from a graduated 1-ml pipette.

The procaine and sodium chlorides are dissolved in the solution.

The volume is made up to 20 ml.

**6 Labels**

Assuming the same conditions of supply as in Example 23.3

**PROTECT FROM LIGHT**

20 ml
<b>PROCAINE AND ADRENALINE</b>
<b>INJECTION B.P.</b>
Procaine hydrochloride 2%
Adrenaline 1 in 50,000
<b>POISON</b>
<b>Bactericide 0.1% w/v Chlorocresol</b>
Sterilised 1 11 64 (3) Name

It might be argued that as the B P gives a complete formula in which the presence and amount of chlorocresol are clearly stated the use of the letters 'B P.' on the label makes any reference to the bactericide unnecessary. Nevertheless, one of the reasons for giving the preservative on the label is to indicate immediately to the user that the preparation must not be given into the spinal fluid or, in large volumes, intravenously and this purpose would not be served by the letters alone.

### 7 Preparation of the Solution

A suitable procedure can readily be devised from the calculation of this example, and the method of preparation of the previous one.

### 8 Filtration

Gravity or vacuum can be used (see Example 23.3) but the latter is preferable for this injection because it reduces the time of exposure of the adrenaline to the atmosphere.

Other stages are as for Example 23.3 except for—

### 9 Sterilisation

Preferably this should be carried out in a steamer. The bottle is put into the basket, the lid put on and the water heated to boiling from cold, after which an exposure of 30 min is adequate.

### EXAMPLE 23.6

*Sterilisation method* Dry heat  
*Type of injection* Single dose of small volume  
*Example* Six 2-ml ampoules of Oily Phenol Injection B P C.

1 This is a simple solution of phenol in almond oil. The dose volume is larger than the maximum of the *British Pharmaceutical Codex* but reference to the *Extra Pharmacopœia* Vol I (24th Ed.) confirms that 2 ml is not unusual.

2 No additions are required. Even if this injection was dispensed in a multiple dose container the concentration of phenol is already much more than the normal bactericidal concentration.

#### 3 Container

A gas tight container is desired, an ampoule cannot be bettered.

#### 4 Overage

This is a viscous injection and, therefore, the overage for a 2 ml ampoule is 0.25 ml.

#### 5 Formula

The minimum volume necessary is  $6 \times 2.25 = 13.5$  ml, but manipulation losses will be heavier than for a mobile injection and, to allow for this and spare ampoules, 20 ml should be prepared.

	B P C	Amounts	Amounts used
Phenol		5 G	1 G
Almond oil	to	100 ml	20 ml

(continued overleaf)

*Example 23 6 continued*

### 6 Labels

Assuming conditions of supply as for the previous three examples—

#### Ampoule

<b>OILY PHENOL</b> <b>INJECTION B P C</b> <hr/> 5% (100 mg in 2 ml)
--

#### Box

Six Ampoules <hr/> <b>OILY PHENOL INJECTION B P C</b> <hr/> 5% (100 mg in 2 ml) <hr/> <div style="border: 1px solid black; padding: 2px; display: inline-block;"> <b>POISON</b> </div> Sterilised 4 6 64 (1) Name
--

### 7 Preparation of the Solution

All apparatus must be dry because traces of moisture make the injection cloudy

Graduate a 50-ml conical flask at 20 ml and thoroughly dry it afterwards. A larger flask is unsuitable because of the difficulty of taring it accurately, 20 ml would be near to the wide bottom and a slight error in placing the mark would greatly affect the volume finally measured.

Put in about 15 ml of oil, add the medicament and dissolve with as little heat as possible, because phenol is very volatile. Cool the solution and make it up to volume.

Phenol is corrosive and should be weighed on a glass watch glass taking care not to spill it on the balance, bench or hands. It can cause a most painful burn and if spillage accidentally occurs it must be cleaned up at once.

### 8 Filtration

Vacuum-assisted filtration is necessary, oils filter too slowly by gravity. A suitable apparatus for a small volume is a jacketed grade-3 sintered glass filter because warm water can be passed through the jacket to speed filtration. Pre-warming the solution is helpful but, in this case, is limited by the volatility of the medicament.

### 9 Filling and Sealing

A wide filling needle is necessary, e.g. one of 20 S W G, and, therefore, the necks must be a little shorter than for a mobile injection, to reduce the risk of contamination with oil, particularly when the needle is withdrawn.

### 10 Sterilisation

After the preliminary inspection for particles and bad seals the ampoules are put in the hot air oven and the procedure given in the section on dry heat sterilisation is followed. The lag time will be negligible in ampoules of this size if they are loaded when the oven is cold.

### 11 Testing the Seals

The simplest method is to immerse the ampoules in the dye solution, heat to boiling and then allow to cool. Heating is necessary because the ampoules will not be hot enough to draw in a significant volume of dye, when removed from the oven. Even if the dye does not dissolve in the oil the entry of the aqueous solution is detectable by the presence of large globules or cloudiness.

The rest of the procedure is as for Example 23.1

#### EXAMPLE 23.7

*Sterilisation method* Dry heat

*Type of injection* Multiple dose

No specific example will be described because the preparation of this type of injection is uncommon. There are two main problems—

#### 1 The Bactericide

All the bactericides recommended for oily injections by the *British Pharmacopœia* are volatile and, therefore, sterilisation must be carried out in a sealed container.

#### 2 The Closure

Closures of natural rubber cannot be sterilised by dry heat and are swollen by contact with vegetable oils. Synthetic rubbers that can withstand one or other of these conditions are available but types resistant to both are not in general use.

The preparation can be sterilised in a screw-capped bottle of the McCartney type with a cap liner of a heat resisting rubber such as silicone; the oil resistance of this polymer is not good and the injection should be kept from the neck of the container by filling through a funnel and using a bottle several sizes larger than necessary.

After sterilisation, the injection is aseptically distributed into sterile, dry multi dose containers which are sealed with an oil resistant rubber (e.g. chloroprene) cap that has been moist heat sterilised and dried by gentle heat.

## INJECTIONS INVOLVING SPECIAL PROCEDURES

### A Use of Minimum Size of Container (e.g. Adrenaline Injection B.P.)

Alkaline, neutral and weakly acidic solutions of adrenaline are very unstable and rapidly lose activity when exposed to oxygen and heat. Oxidation leads to a change of colour through pink to brown but heat decomposition takes place by other routes and the products may not be coloured. In both cases pH is a very important influencing factor.

Adrenaline is only sparingly soluble in water and

the first official solution of adrenaline for injection contained the hydrochloride, the salt being made from the base and acid during the preparation of the solution.

Work by Berry and West (1944), West (1945, 1946) and Foster, McDougall and Thorpe (1945) led to the replacement of the hydrochloride by the tartrate with the following advantages—

1 Greater stability Under the conditions listed below, the solution can be heated in sealed ampoules

for 5 hr at 115°C with only 30 per cent loss of activity. Probably the tartrate is more stable because it is weakly ionised compared with the hydrochloride, i.e. the adrenaline is held in protective combination.

2 Adrenaline acid tartrate is a crystalline salt, readily soluble in water, and, unlike adrenaline itself, it keeps indefinitely in air. Therefore, the separate use of base and acid is unnecessary.

In the present injection, oxidative changes are prevented by—

(a) Adding a reducing agent (0.1 per cent sodium metabisulphite)

(b) Using well filled, well-closed containers—ampoules are preferable

(c) Protection from light

(d) Ensuring a pH of about 3.6. The injection contains the equivalent of 1 in 1,000 of adrenaline base and it is fortunate that the amount of acid tartrate needed to provide this produces, with the metabisulphite, a solution of pH slightly under 3.7. This falls a little during autoclaving and, consequently, the preparation is at the optimum value and does not need adjustment.

The most powerful factor controlling the heat change is pH and in this case, also, maximum stability is shown at 3.6, or slightly lower.

The B.P. gives limits of 3.2 to 3.6. Since pHs on the alkaline side of this range are most harmful, it is necessary to use glass complying with the limit for alkalinity.

West's work (see also, 1950) has continually emphasised the importance of well-filled containers. The maximum dose of Adrenaline Injection is 0.5 ml, and when ampoules containing this volume are prescribed the correct size must be used. The existence of ampoules smaller than 1 ml is sometimes overlooked, because they are not often required, but for easily oxidised medicaments, where a minimum air space is necessary, it is important to use the smallest possible size.

Rapid deterioration of adrenaline solutions has occurred in yellow ampoules used for light protection (see p. 284). For this reason, and because it is necessary to be able to check easily that the solution is clear and colourless (see the B.P. description) it is preferable to ensure light protection by packing in a box and adding a warning label. Contact with metals should be avoided during preparation.

#### B Nitrogen filling (e.g. Apomorphine Injection B.P.)

Where replacement of air by an inert gas is directed, ampoules should be used because it is impracticable

to prevent the entry of small volumes of air during the withdrawal of doses from a multi-dose container.

Nitrogen is the gas most often used. It is conveniently supplied by a permanent pipe line from a cylinder stored in an annexe. At the bench there should be a fine control valve to which the gas-filling unit can be connected when necessary. This unit (Fig. 23.13) consists of an air filter connected to a long filling cannula (i.e. a needle with its tip ground flat) surrounded by a deep glass hood. If a piped supply is not available, an alternative, suitable for a few ampoules, is a gas-filled football bladder.

It is advisable to use a spare ampoule, of the size used for the injection but filled with water, for adjusting the gas flow. If an ampoule of the injection is used, some of the solution may be blown into, or even out of, the neck if the initial flow is too fast. A suitable flow, which should be set with the bench valve, just disturbs the liquid surface when the

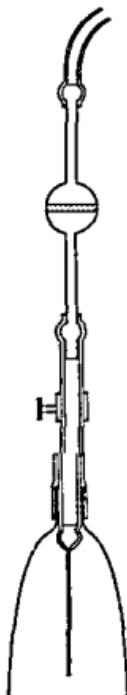


Fig. 23.13 UNIT FOR NITROGEN FILLING

needle tip is about 5 mm above it, and the gate clip, on the tube just above the needle, is fully open. The clip is used to isolate the supply between successive fillings and avoid waste of nitrogen, if the bench control valve was closed instead it would be necessary to readjust the flow rate each time an ampoule was filled.

Ideally each ampoule should have its air replaced immediately after it has been filled and then it should be sealed at once. Replacement time varies with container size but two or three seconds is sufficient for a 1-ml ampoule.

The following precautions help to minimise contact with oxygen during preparation—

- 1 Use small, well-covered vessels
- 2 Avoid vigorous stirring and shaking
- 3 Filter by vacuum to hasten the process but do not exceed a fast drip because much aeration will occur if the solution is allowed to rush through the filter. If pressure filtration is preferred, use nitrogen, not air
- 4 Minimise the time between preparation and sealing

For very easily oxidised substances, such as phenotolamine methanesulphonate, it may be necessary to dissolve, filter and fill under nitrogen. This involves the use of an enclosed system to which nitrogen is supplied under slight pressure. Agitation to accelerate solution can be achieved by bubbling the gas through the solvent.

Solid apomorphine hydrochloride must not be used if an emerald green colour is produced when it is dissolved in 100 parts of water. It must be stored protected from light in well-closed containers. The injection must be colourless, or almost colourless, and a definite green tint is a sign that it is unsafe for use. A warning to this effect should be given on the label. Avoidance of contact with metals is necessary.

#### C. Preparation of a Suspension (e.g. Bismuth Oxychloride Injection B.P.C.)

This is a suspension of bismuth oxychloride in Water for Injection. It also contains 0.9 per cent of sodium chloride, to produce isotonicity, and 0.1 per cent chlorocresol, as a bactericide.

No suspending or dispersing agents are included and, therefore, the heavy bismuth oxychloride must be in a very fine state of subdivision. The B.P.C. requires a very fine powder, and the drug should be purchased in this form or sieved before use.

Because there is no suspending agent the powder sediments quickly and this makes distribution of equal amounts of powder into ampoules rather

difficult. Also, it is not easy to withdraw for administration a dose containing the full amount of medicament.

Provided the user can be relied upon to shake the bottle vigorously there is a case for supplying small batches of this injection in multi-dose containers. Then, preparation can be done in the bottles, thus ensuring that each contains the right amount of powder. However, if the bottle is not properly shaken the residual suspension will become progressively more concentrated as successive doses are withdrawn, and the risk of overdosage will increase accordingly. With ampoules, overdosage cannot occur but underdosage is possible, due partly to the difficulty of shaking such a small container efficiently and partly to the longer delay (caused by the need to open an ampoule) between shaking and withdrawal. Since, in general, overdosage is more serious than underdosage, on balance it is better to use ampoules.

The cartridge is very suitable for a suspension, the dose is transferred directly from container to patient and, provided the correct amount is put into it, underdosage is not possible.

When ampoules are required, the suspension is made in a beaker and a small power driven plastic stirrer is used to stop the powder from settling during filling. Afterwards the ampoules are allowed to stand and then the sediment heights are checked by eye to ensure that they are equal.

A 'Shake' label is necessary, even on the individual ampoules. The box must be labelled 'For intramuscular use only' (see 'Formulation of Injections').

#### D. A Supersaturated Solution (e.g. Calcium Gluconate Injection B.P.)

The usual strengths of this injection are supersaturated solutions but the tendency for crystals to separate can be greatly reduced by replacement of up to 5 per cent of the gluconate with calcium d-saccharate or other harmless salt.

If an unstabilised solution is dispensed precautions are necessary to prevent crystallisation—

1 Protect the mouths of containers during preparation, because dust particles act as nuclei for crystal formation.

2 Filter by vacuum through a sintered glass funnel surrounded with a hot water jacket.

3 Keep the ampoule necks dry during filling because crystals deposited from the solution by the heat of sealing may not dissolve during sterilisation if the ampoule is autoclaved upright, and may later seed crystallisation. Multi-dose containers are

unsuitable because particles from the closure may act as crystal foci.

A warning against the use of an injection in which crystallisation has occurred is necessary on the label, particularly as the preparation is often given intravenously.

#### E. A Soap Solution (e.g. Ethanolamine Injection B.P.C.)

This injection is a solution of the soap, ethanolamine oleate. It is made from ethanolamine and oleic acid. The latter is insoluble in water and the slightest excess causes turbidity in the, normally sparkling and clear, solution. On the other hand, if excess ethanolamine is used the pH may be outside the B.P. limits. Consequently the two liquids must be measured with exceptional care. When only a few ampoules, such as six of the 5 ml size, are prescribed the weights required contain figures at the third decimal place. If a rider is available, this presents no problem, apart from the fact that if only the right hand beam is calibrated it will be necessary to weigh on the left hand pan. In the absence of a rider 100 ml should be made to avoid approximations which might lead to a cloudy or alkaline product. This large excess is permissible, partly because it ensures a satisfactory product and partly because, in practice, it would be used to make additional ampoules which, since the injection is quite stable, could be stored until required. If the ethanolamine and oleic acid, both of which are liquids, are added to the weighing container from capillary pipettes, accurate control of delivery is facilitated and weighing errors are much less likely.

For a volume as small as 100 ml, the following method is more successful than the one in the Codex.

1 Put 25 ml of water into a 100-ml stoppered cylinder or a glass-stoppered bottle, tared at 100 ml.

2 Weigh the oleic acid, transfer to the cylinder and thoroughly rinse the container used for weighing. The type of weighing boat shown in Fig. 23.14 is particularly convenient as it is easily emptied and rinsed through the hollow stem. At this stage the volume, with the rinsings should be about 50 ml.

3 Add the benzyl alcohol from a bulb pipette



Fig. 23.14 WEIGHING BOAT

4 Weigh the ethanolamine, transfer to a conical flask containing about 10 ml of solvent and thoroughly rinse the scoop into the same container. Failure to transfer the ethanolamine completely is the commonest cause of cloudiness because, then, part of the oleic acid is left uncombined. Make up the volume to about 35 ml.

5 Shake the contents of the cylinder thoroughly, to dissolve the benzyl alcohol and reduce the oleic acid to fine globules that will present a large surface to the ethanolamine and, therefore, react with it quickly.

6 Add all the ethanolamine solution and mix by tipping the liquids rapidly from end to end of the cylinder. During the mixing add rinsings from the flask. If the cylinder is shaken vigorously a large amount of froth is produced which, because it contains entrapped solution, must be allowed to subside before making up to volume. A very long time is necessary for several inches of froth to disappear. Dilution of the ethanolamine and early addition of the benzyl alcohol prevent the formation of a very viscous, concentrated soap, lumps of which are sometimes difficult to disperse.

The completed injection froths easily if roughly handled and if air is sucked through it during filling. This froth complicates accurate measurement of ampoule volumes and, therefore, the solution should be drawn into the syringe slowly, taking care meanwhile to keep the needle-tip well below the surface of the bulk liquid. If these precautions are ignored the ampoules may receive startlingly different volumes. Gentleness is also necessary when transferring the injection from syringe to ampoule, to prevent froth rising into the neck. After filling, the ampoules should be placed on a flat surface and the liquid levels critically compared.

The concentration of benzyl alcohol is well in excess of that used when this compound is added as a bactericide, consequently no further addition is needed when the injection is supplied in multi-dose containers. The soap and pH are also inhibitory to micro-organisms.

The label must bear the warning 'For intravenous use only, as a sclerosing agent' because this type of preparation, intended to irritate the inner wall of a vein, can produce severe damage, such as ulceration, if injected into the surrounding subcutaneous tissue.

#### F. pH Adjustment (e.g. Mersalyl Injection B.P.)

This injection is sterilised by Heating with a Bactericide. Phenylmercuric nitrate must be used as the

bactericide, it is the obvious choice for an injection containing a mercury derivative.

It is one of very few injections for which there is a definite official direction to avoid contact with metals. This is because ions of other metals may displace toxic mercury ions from the complex.

It must be packed in ampoules because extractives from the rubber closures of multi-dose containers may cause decomposition.

Consider the preparation of six 2-ml ampoules.

#### Formula

	B P	Amounts	Amounts used
Mersalyl acid	9.56 G	2.39 G	
Theophylline	5 G	1.25 G	
Sodium hydroxide	1 G or q s	0.25 G	
0.002% w/v Phenyl-mercuric nitrate solution	to 100 ml	to 25 ml	

The preparation of 25 ml avoids a third decimal place in the weight of the acid, the extra volume can be used for the final pH check.

Fill a 5-ml burette with a 10 per cent solution of sodium hydroxide in the solvent, approximately 2.5 ml will be needed. A burette is the most convenient apparatus for making successive additions of small volumes of neutralising solutions in pH adjustments.

Add the mersalyl acid and theophylline to 20 ml of the solvent in a covered 100-ml conical flask and then add the sodium hydroxide solution drop by drop, rotating the flask vigorously after each addition and applying gentle warmth until the ingredients have dissolved.

Continue to add alkali until the pH is 8.0. At first it is convenient to use bromothymol blue indicator on a tile. The first full blue indicates a pH of 7.6. At this stage continue with a thymol blue capillary until the greyish colour indicative of pH 8 is obtained.

Clarify and make up to volume through the filter. Then check the pH with a meter, using the minimum amount of filtrate and rejecting this afterwards. Readjust if necessary, at most, only a trace of alkali will be needed and this will not significantly affect the strength of the injection.

#### G. Saturation with Carbon Dioxide (e.g. Sodium Bicarbonate Injection B.P.)

The reason for treating this injection with carbon dioxide before it is sterilised by autoclaving was discussed under the 'Formulation of Injections'.

The solution is simply prepared by dissolving the bicarbonate in water and filtering carefully. It is

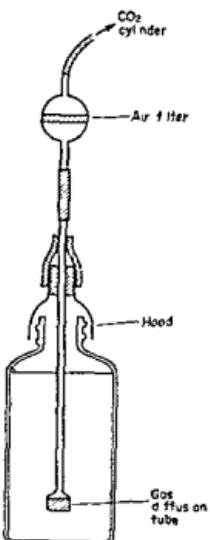


Fig. 23.15 APPARATUS FOR PASSING CARBON DIOXIDE

administered intravenously for the correction of acidosis.

One satisfactory way of passing carbon dioxide is to connect a cylinder of the gas to a gas diffusion tube (Fig. 23.15) which has a sintered end of grade-2 porosity that produces fine streams of bubbles. In the connecting tubing there should be an air filter to remove dust from the gas (it is *inadvisable* to rely on the coarse diffusion sinter) and a glass hood to keep air-borne particles out of the bottle.

In addition to saturating the solution with gas, the passage of carbon dioxide also converts the carbonate impurity in the medicament into bicarbonate, even 'Analar' material may contain as much as 1 per cent of carbonate. Michaels (1948) pointed out that the time for complete conversion depends on the gas flow rate, the volume of the solution and the concentration of bicarbonate and, therefore, of carbonate. For example, he found the B.P. method of passing gas for 1 minute inadequate for 1-pint volumes of 1 per cent and  $\frac{1}{2}$ -pint and 1 pint volumes of both 5 and 8 per cent solutions. Consequently, he recommended pH determination (using either a meter or an indicator) instead of passage of the gas for a prescribed time, as a more accurate means of ensuring adequate treatment with carbon dioxide. Carbonate is absent if the pH is 8.35 or less or the

injection does not colour phenolphthalein solution (indicating a pH of 8 or less). If either of these methods is used samples of the injection must be removed from the bottle for testing and allowance for this should be made by including a sufficient excess.

Michaels also investigated the time for restoration of the original bicarbonate concentration after autoclaving (for 1 hr)—

*Time after sterilisation      Original bicarbonate (%)*

Immediately	92
3 hr	95
24 hr	almost 96

The B.P. statement that bottles must not be opened until at least 2 hours *after cooling to room temperature* should ensure a reversal of about 95 per cent.

In bottles only  $\frac{1}{2}$  full he found only 89 per cent of the pre sterilisation concentration after 24 hr, indicating that containers should be as full as possible. He claimed that bottles could be  $\frac{1}{8}$  full without risk of bursting. Transfusion bottles are suitable if their closures are perfect and are screwed on as tightly as possible as soon as the gas flow is stopped.

Sometimes this injection is dispensed in ampoules. Then, a unit similar to that used for nitrogen filling is suitable but the needle must be longer because the gas must pass into the solution.

Bacterial filtration is allowed as an alternative to autoclaving and although saturation with carbon dioxide is not required officially it could be helpful for converting the carbonate impurity into bicarbonate. Filtration should not be assisted by vacuum because this causes bicarbonate solutions to lose carbon dioxide with production of carbonate (Michaels).

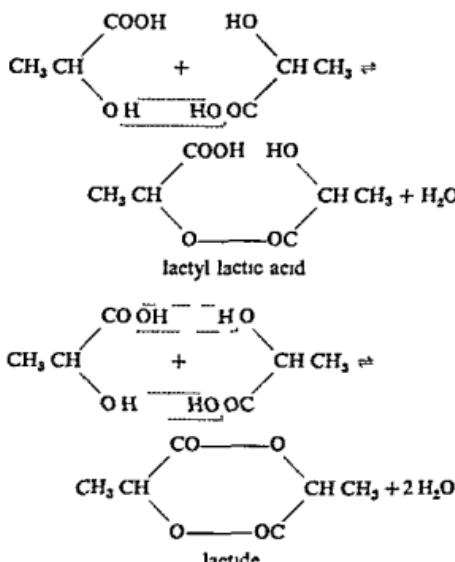
An isotonic solution of this injection contains about 1.4 per cent of sodium bicarbonate.

#### H Compound Sodium Lactate Injection

This injection has two important synonyms—Hartmann's Solution for Injection and Ringer-Lactate Solution for Injection. It is dispensed in large volumes, which may be administered intra venously or by mouth. The latter route is used in young children suffering from severe dehydration as a result of persistent vomiting and diarrhoea; in such patients it is often impossible to find a suitable vein to use for infusion because of the loss of fluid from the circulation. When the solution is given orally, purified water is a satisfactory solvent because a pyrogenicity is not essential, nevertheless, it must be packed as an injection and sterilised because the

lactate ion supports the growth and multiplication of many micro-organisms, and seriously ill children are in no condition to combat additional infection.

The sodium lactate is made from lactic acid and sodium hydroxide during the preparation of the injection. However, this is complicated because lactic acid always contains a proportion of anhydrides which are readily produced in the following ways—



These must be hydrolysed back to lactic acid before formation of the lactate is possible. The hydrolysis is achieved by autoclaving in the presence of a slight excess of the alkali. Afterwards the excess is neutralised with dilute hydrochloric acid (Hopper and Jones, 1956).

The preparation of 500 ml of the injection will be discussed.

#### Formula

	B.P. amounts	Amounts used
Lactic acid	2.4 ml	1.2 ml
Sodium hydroxide	1.14 G	0.57 G
Dilute hydrochloric acid	g.s.	g.s.
Sodium chloride	6.0 G	3.0 G
Potassium chloride	0.4 G	0.2 G
Calcium chloride	0.4 G	0.2 G
Water for Injection	to 1,000 ml	to 500 ml

1 Put 100 ml of the solvent into a 250-ml McCartney bottle

2 Weigh the sodium hydroxide as quickly as possible to minimise absorption of moisture by the deliquescent pellets. If a significant amount of the weight is due to water there may not be sufficient alkali to leave an excess after heating. The film of carbonate on the surface of the pellets also reduces the neutralising power of the alkali because weight for weight it is less efficient than hydroxide. If, even after rapid and careful weighing, the solution is acid after autoclaving a new sample of sodium hydroxide should be used on the next occasion. Rinse all traces from the balance pan into the bottle.

3 Add the lactic acid from a pipette

4 Seal the bottle and autoclave at 115° to 116°C for 1 hr

5 While the heating is in progress, dissolve the other ingredients in 350 ml of solvent. The calcium chloride is also deliquescent and must be weighed rapidly. Immediately replace the stopper of the stock bottle.

6 Set up a 1- or 2 ml microburette and fill it with dilute hydrochloric acid

7 Thoroughly clean a white tile and two glass rods and prepare a phenol red capillary for use

8 After the heating period remove the bottle from the autoclave, with the usual precautions, and allow it to cool without assistance

9 Put a drop of solution on the tile with one rod, add a drop of indicator with the other, and mix thoroughly. It is more satisfactory not to put the phenol red on the tile first because carbon dioxide and other atmospheric gases may affect it if there is

delay in adding the injection. The first check should be made before any acid is added because occasionally there is no, or very little, excess alkali and the colour obtained will give some indication of the amount of acid that can safely be added at once. In any case, only a few drops should be added before re-testing. Make additions and tests until a change towards orange is obtained, then continue with the capillary until a definite orange (pH 7.0) is produced. It is quicker to use the tile but the more accurate capillary method should be used near to the end point. Take care to use clean glass rods and capillary tubes.

10 Mix the lactate and chloride solutions, filter, pack and sterilise immediately. Because of the ability of this solution to support the growth of micro-organisms, 'immediately' should be narrowly interpreted.

11 If the preparation is for oral use and has been made with purified water it should be labelled 'Compound Sodium Lactate Solution' with a prominent additional warning 'For oral use only'.

When this injection is intended for intravenous infusion the label must state the strength in mEq/litre, i.e. calcium 4, potassium 5, sodium 131, bicarbonate (as lactate) 29 and chloride 111, a total of 140 mEq each of anions and cations. Therefore, the solution is slightly hypotonic with respect to blood plasma. It could be made isotonic, if required, by an appropriate increase of the sodium chloride content. The reference to bicarbonate, instead of lactate ion, is because the latter is converted into the former in the body.

*See also Sykes (1959)*

## EXERCISES

### Calculations

Set down the formulae for the following in the form suggested in this chapter and then work out the amounts of medicaments, and the triturations and dilutions required to prepare the final solutions.

1 Six 1-ml ampoules of Apomorphine Injection B P

2 Six 10-ml ampoules of Calcium Gluconate Injection B P containing the maximum amount of stabiliser

3 15 ml of Morphine Sulphate Injection B P containing 20 mg of morphine sulphate per ml

### Labels

Write labels for the following. Unless the word 'Dispense' is used assume that supply is other than on a prescription. Use actual labels to obtain practice at including all the requirements in a small space.

1 Dispense six 2 ml ampoules of Sterile Strong Noradrenaline Solution B P C

2 Dispense six 20-ml ampoules of Calcium Gluconate Injection B P containing the maximum amount of calcium d saccharate as stabiliser

3 Send 500 ml of Compound Sodium Lactate Injection B P, for use by intravenous infusion

4 Prepare 10 ml of Deoxycortone Acetate Injection B P

*Exercises continued*

## Calculations

- 5 20 ml of Procaine and Adrenaline Injection B P
- 6 Six 2 ml ampoules of Dimercaprol Injection B P.
- 7 Six 1-ml ampoules of Cyanocobalamin Injection B P
- 8 Six 1-ml ampoules, each containing—  
Morphine sulphate 15 mg  
Hyoscine Hydrobromide 0.5 mg  
Water for Injection to 1 ml
- 9 Six 4-ml ampoules of Sterile Strong Noradrenaline Solution B P C
- 10 Six 1-ml ampoules of Carbachol Injection B P

## Labels

- 5 Prepare 20 ml of Lignocaine and Adrenaline Injection B P.
- 6 Dispense six 1-ml ampoules of Quinine Hydrochloride Injection B P
- 7 Dispense six 1-ml ampoules of Pethidine Injection B P containing 50 mg/ml, for use in a maternity ward of a hospital
- 8 Prepare 20 ml of Procaine and Adrenaline Injection B P
- 9 Dispense six 1-ml ampoules of Tubocurarine Injection B P
- 10 Send six 10-ml ampoules of Sterile Potassium Chloride Solution B P

## REFERENCES

- 1 ARTZ, W J, GLOOR, W T and REESE, D R (1961) Study of various methods of detecting leaks in hermetically sealed ampoules *J Pharm Sci* 50, 258-262
- 2 BEAN, H S and WALTERS, V (1954) Storage of ampoules in phenol solutions *Pharm J* 173, 465-466
- 3 BERRY, H and WEST, G B (1944) The stability of adrenaline solutions Part 1 Solutions of adrenaline hydrochloride *Quart J Pharm* 17, 242-248
- 4 BLENIKOV, C H (1953) A simple ampoule washer *Pharm J* 170, 400
- 5 BOEIM, E M (1955) Work in a Swiss hospital *Pharm J* 174, 76
- 6 BRINDLE, H and RIGBY, G (1946) The preparation of non pyrogenic water and infusion fluids, using activated charcoal *Quart J Pharm* 19, 302-309
- 7 BRITISH STANDARD 1752 1963 Laboratory sterilized or filtered filters British Standard Institution, London
- 8 CASE REPORT (1953) Perculation of disinfectant into ampoules *Pharm J* 171, 396
- 9 COOPER, J W and GUNN, C (1957) *Tutorial Pharmacy* 5th Ed Pitman, London
- 10 COOPER, P (1951) A scheme for intravenous solutions Part 1, Containers *Pharm J* 166, 86
- 11 COOPER, P (1952) Safety in the preparation of injections *Pharm J* 169, 336
- 12 COPE, R W, PRESTCOTT, F and WHITTET, T D (1952) Injection routine in operating theatres *Pharm J* 168, 222
- 13 DARWIN, K V and DEE, G M (1957) Experiences with a PVC filter medium. *Pharm J* 179, 133-134
- 14 DAVIS, H (1946) *Quart J Pharm* 19, 410
- 15 DAVIES, E V and MILLAR, M L (1948) Useful apparatus *Pharm J* 160, 168-169
- 16 ETCHILLS, D (1951) Small scale preparation of solutions in ampoules *Pharm J* 167, 152-153
- 17 FORD, T B, LTD (1960) *Sterumat supplement No 2 Pyrogens* Filtration Sales Division, E.C.4 p 4
- 18 FOSTER, G E, McDougall, A C, THORPE, R H (1945) An improved solution of adrenaline prepared from adrenaline tartrate *Quart J Pharm* 18, 279
- 19 FOWLER, H W (1960) *Aids to forensic pharmacology* 5th Ed Balliere, Tindall and Cox, London
- 20 FOWLER, F S (1959) Cleaning of glassware and rubber closures (1959) *Publ Pharm* 16, 97-100
- 21 GILLANDERS, K (1956) An ampoule box for ward use *Alchemist*, Leeds 20, 193
- 22 CODDING, E W (1945) Foreign matter in solutions for injections *Pharm J* 154, 124-125
- 23 GOSBY, W C and HANSON, J C H (1950) Filtration apparatus for parenteral and other solutions *Pharm J* 165, 417-418
- 24 GRAHAM, W D, CHATTEN, L G, PEVAROWSKI M COX, C E and AIRTH, J M (1959) A collaborative study of the detection of particles in ampouled solutions *Drug standards* 27, 61-76
- 25 HOPPS, C and JONES, B (1956) Sodium lactate for injection. *Pharm J* 177, 36

- 26 HUDSON, F (1944) Activated charcoal in the preparation of intravenous solutions *Pharm J* 152, 131-132
- 27 HUDSON, H E (1961) A method of measuring matter suspended in liquids *Pharm J* 186, 201-203
- 28 MICHAELS, I (1948) The sterilisation of sodium bicarbonate solutions *Quart J Pharm* 21, 231-238
- 29 MIN OF FOOD (1954) *Food standards committee report on colouring matters* Her Majesty's Stationery Office, London p 27
- 30 MIN OF FOOD (1955) *Food standards committee, Supplementary report on colouring matters* Her Majesty's Stationery Office, London p 15
- 31 MYERS J A (1948) Preparation of intravenous solutions *Pharm J* 160, 9 10
- 32 NEWMAN, F H (1954) Sintered stainless steel filters M and B *Pharmaceutical Bulletin* 3, 140-141
- 33 NORMAN, R (1951) Visual examination of drip solutions *Pharm J* 167, 342
- 34 PATEL, C M (1955) The labelling of ampoules *Pharm J* 175, 580
- 35 RESUGGAN, J C L (1957) *The cleaning and sterilisation of bottles and other glass containers* United Trades Press, London
- 36 SCOTT, P G W (1952) An apparatus for the determination of volumes of injections in single dose containers *J Pharm Pharmacol* 4, 139-140
- 37 SHEPLEY, G F (1950) The importance of labels *Pharm J* 165, 124-125
- 38 SMITH, K L (1954) Symposium on pyrogens (discussion) *J Pharm Pharmacol* 6, 325
- 39 SYKES, C H (1958) Filtration *Publ Pharm* 15, 163-165
- 40 SYKES, C H (1959) Some intravenous transfusion solutions for special purposes *Publ Pharm* 16, 178-180
- 41 THOM, A S (1950) Apparatus for low pressure filtering *Pharm J* 164, 225
- 42 WEST, G B (1945) The stability of adrenaline solutions Part II *Quart J Pharm* 18, 267
- 43 WEST, G B (1946) The stability of adrenaline solutions, Part III *Quart J Pharm* 19, 256-259
- 44 WEST, G B (1950) Stability of solutions of adrenaline tartrate *J Pharm Pharmacol* 2, 864
- 45 WHITTET, T D (1954) The occurrence and importance of pyrogens *J Pharm Pharmacol* 6, 304-309
- 46 WOOD, D G (1952) A simple ampoule washing device *Pharm J* 168, 159



## Aseptic Technique

THE aim of aseptic technique is to prevent the access of micro-organisms during the preparation and testing of pharmaceutical products

### Preparation

Aseptic technique is unnecessary if the last two stages in the processing of a sterile product are—

- 1 Packing in a container and sealing to prevent contamination after sterilisation, followed by
- 2 Sterilisation by one of the three official heat-sterilisation processes—dry heat, autoclaving or Heating with a Bactericide

The terminal heat treatment and reliable packing ensure the issue of sterile preparations

However, there are several classes of product for which a terminal heat treatment is not possible, for example—

(a) *Thermolabile Soluble Substances, Stable in Solution* Solutions of these can be filtered through a bacteria proof filter but aseptic technique is required to prevent contamination of the filtrate during collection and while it is being packed and sealed in the final containers. An example is Aneurine Hydrochloride Injection

(b) *Thermolabile Soluble Substances, Unstable in Solution* Filtration through a bacteria-proof filter is impracticable because these medicaments are not stable in solution sufficiently long. They must be dissolved aseptically in a sterile solvent just before use, e.g. Neoarsphenamine Injection

(c) *Thermolabile Suspensions, Stable in the Vehicle*, e.g. Propylidone and Propylidone Oily Injections

(d) *Thermolabile Powders that require Dilution with Other Powders*, e.g. Penicillin and Sulphathiazole Dusting Powder

(e) *Thermolabile Powders that require Incorporation in a Semi solid base*, e.g. the eye ointments of the British Pharmacopoeia

Filtration is impossible in the last three cases and therefore, the medicament must be mixed with the appropriate sterile vehicle aseptically

### Testing

Aseptic technique is also necessary in sterility testing. Growth in a nutrient medium inoculated with a sterile product can be attributed to contamination of the product only if the manipulations involved in performing the test are faultless

## SOURCES OF CONTAMINATION

Satisfactory rules for good aseptic technique can only be devised if the possible sources of contamination are fully appreciated

### 1. The Atmosphere

The atmosphere has no flora of its own because it cannot support the growth of micro-organisms. Nevertheless, as the appearance of a shaft of light in a darkened room impressively demonstrates, it is usually heavily contaminated with particles. Micro-organisms are associated with many of these

### TYPES OF CONTAMINATION

#### (a) Dust

A high percentage of the dust particles in outside air come from the soil and, therefore may carry soil bacteria. These are chiefly saprophytes and include cocci (mainly species of *Sarcina* and *Micrococcus*) and sporing rods (particularly *Bacillus* spp), but pathogenic anaerobic sporing rods (e.g. *Clostridium tetani* and *Clostridium welchii*) are also quite common.

Indoors the dust stirred up by cleaning operations sometimes contains resistant pathogens from infections suffered or carried by recent occupants of the room. Examples are *Staphylococcus aureus*, which may remain viable in dust for months,  $\beta$ -haemolytic streptococci and, less often, *Mycobacterium tuberculosis* and intestinal bacteria.

#### (b) Droplets

Large numbers of droplets are expelled from the respiratory tract by coughing and sneezing and may contain organisms from the nose, mouth, throat and lungs. Healthy carriers often distribute *Staphylococcus aureus* and  $\beta$ -haemolytic streptococci in this way and the transfer of the common cold, influenza, the virus diseases of childhood and tuberculosis by droplet infection is well known.

#### (c) Droplet Nuclei

The smaller droplets evaporate quickly, and as they contain saliva or mucus the residue consists of tiny protein flakes carrying any organisms previously in suspension.

#### (d) Free Micro-organisms

Bacteria and viruses free from dust droplets or nuclei are uncommon but naked yeasts and mould spores are often abundant.

### FATE OF THE CONTAMINANTS

#### (a) Dust

The rate at which dust settles from the air depends, among other factors, on the particle size, the amount of air movement and the humidity. Most particles in still outdoor air are between 0.1 and 1  $\mu$  in size and, therefore, are small enough to be retained almost indefinitely by Brownian movement. The latter begins to interfere significantly with gravitational fall at a particle size of about 3  $\mu$  and predominates at less than 0.1  $\mu$ . The figures in Table 24.1, selected from a table by Davies (1952) show the sedimentation rates of individual spherical particles.

Table 24.1

Diameter ( $\mu$ )	Rate of fall (cm/sec)
0.2	0.000225
1	0.0035
10	0.3
100	25
1,000	385

i.e. a 1  $\mu$  particle takes about an hour to fall 12.5 cm.

of unit density in air at 20°C and one atmosphere pressure.

If the air is disturbed by wind, draughts or movements of occupants, sedimentation is reduced, fresh dust becomes airborne and the lighter particles are spread over a much wider area.

#### (b) Droplets

Coughing and sneezing produce droplets of from 1 to 2,000  $\mu$  but most are under 100  $\mu$ . The large ones sediment rapidly quite close to the point of production but those smaller than 100  $\mu$  evaporate quickly and become droplet nuclei before their fall is complete. The evaporation rate depends on the temperature and humidity of the atmosphere and the surface area of the droplet. Since droplets get smaller during evaporation, their surface area, relative to their volume, increases and, therefore, the evaporation rate increases too. Droplets of 100  $\mu$  take only 1.7 sec to evaporate completely at 18°C.

#### (c) Droplet Nuclei

These are so small and light that they remain suspended and quickly become spread over a wide area by air currents.

#### (d) Free Micro-organisms

Since cocci are about 1  $\mu$  in diameter and sporing rods average about 5  $\mu$  in length they sediment slowly in still air but are kept suspended by air movements.

### 2 The Breath

In normal breathing few organisms pass into the atmosphere because they are retained in the fluids lining the respiratory tract, but coughing, sneezing and spitting can cause contamination at considerable distances by expelling droplets of these fluids.

Haemolytic streptococci and *Staphylococcus aureus* are present in the noses and throats of a high percentage of the population. For example, *Staphylococcus aureus* is found just inside the nostrils, and, therefore, very favourably positioned for distribution, in about 20 per cent of healthy adults regularly and, considerably more, intermittently.

### 3 The Hands

These are a major means of transmitting infection. It has been estimated that there are not less than 10,000 organisms per  $\text{cm}^2$  of normal skin (quoted by Walter, 1954). These organisms fall into two groups—

(i) *The Resident Flora*. This consists of bacteria that can live and multiply on the surface of the skin.

or in the hair follicles and the ducts of the sebaceous glands. They are mostly non-pathogens, but *Staphylococcus aureus* is occasionally found.

(ii) *The Transient Flora* This is composed of organisms collected from the environment or from other parts of the body, e.g. the fingers may be contaminated by blowing the nose or through inadequate post toilet washing. These are often killed quickly by the antibacterial activity of the unsaturated fatty acids of the sebaceous glands or by the dryness of the skin compared with their normal habitat.

#### 4 Clothing

Part of the atmospheric dust that becomes entangled in the fibres of fabrics is dislodged by body movements and can considerably raise the level of contamination around a person who is working carelessly. A special danger is the load of contami-

nated particles shed from a handkerchief that has dried after previous use.

#### 5 The Hair

Hair is constantly exposed to atmospheric dust which it entangles and may liberate during such motions as shaking the head and brushing a lock from the face.

#### 6 The Working Surface

This is a potential source of contamination because organisms will sediment on to it from the air.

#### 7 Equipment

In aseptic technique no source of contamination is more serious than unsterile equipment.

(Further information on sources of infection is contained in McDermott (1961), M.R.C. Memo (1951), M.R.C. Report (1948) and Williams *et al.* (1960).)

### THE DESIGN OF AN ASEPSIS LABORATORY

The requirements for aseptic technique need not be as strict in a small hospital, where one worker occasionally performs a simple process such as adding a sterile solvent to several containers of a sterile antibiotic, as in a manufacturing house that employs a team of women full time, in one large room, on, for example, the more complicated procedure of packing sterile powders from bulk into individual vials. Also, on a small scale, economic considerations play a far more important part in influencing the choice of particular design features. These factors should be borne in mind as the following information is studied because it does not follow that the most efficient methods are always essential or economically practicable.

#### A Site

Attempts to maintain aseptic conditions are more likely to succeed if staff are able to work undisturbed. Usually this can be ensured by appropriate education of everyone who works in or regularly visits the department but, as a precaution, it is advisable to site the asepsis laboratory as far as possible from the rooms to which non pharmaceutical staff have access.

In hospitals, the possibility of transmission of infection from wards should be taken into account although the recent emphasis on the prevention of cross infection has lowered this risk. If practicable, the laboratory should be away from stairs, lift shafts and corridors all of which provide routes by which airborne micro-organisms can travel about the building.

The relationship of the laboratory to the other rooms of a sterile products suite is important. Since an asepsis room should contain few or no storage facilities, the sterile equipment and products store must be adjacent or near by. Access to the laboratory should be through one or more rooms with washing and changing facilities. Space for records, label writing, inspection and finishing will be required but it may be possible to use the accommodation available for heat-sterilised products.

#### B Size

The main factor controlling the size of the room is the maximum number of people using it at any one time. Apart from this, a large, fairly high room is more pleasant to work in, and the overall level of micro-organisms in its atmosphere is less affected by local air disturbances or contamination produced by individual workers.

Conversely the capital and maintenance costs of the equipment for controlling the microbial content, temperature and humidity of the atmosphere are reduced by keeping the room small. Cleaning the upper walls and ceiling, often neglected if they are difficult to reach, is made easier if the ceiling is low.

Sometimes, when the amount of aseptic dispensing is insufficient to keep the laboratory in continuous use, it is also used for preparing heat-sterilised injections, including infusion fluids. The clean atmosphere greatly assists the production of particle free solutions. In these circumstances the room must be much bigger.

### C. Windows

Because of the dust-collecting and dust-distributing propensities of windows it is tempting to do without them altogether but, even if the artificial lighting is excellent, it is not pleasant to work day after day in a windowless room, and efficiency is likely to suffer. Also, bright sunshine is the best detector of dust.

Daylight can be provided by areas of glass bricks, which can be built flush with the inner wall of the room to dispense with a dust-collecting sill, but it is difficult to avoid rough crevices between the individual bricks. They are not easily broken but they are scarcely more popular with staff than artificial light, because it is not possible to see through them.

Large windows of clear glass are most acceptable to staff but they must not open, and ventilation should be provided by an air-filtration system. The heat losses that occur from extensive areas of glass can be reduced by double glazing, the inner sheet being fixed and the outer one made openable for cleaning. Shading from the sun in summer can be given by a remote-controlled venetian blind within the space. The inner window should be a single pane and its frame must have no unnecessary architraves.

### D Doors

If possible, the laboratory should be entered through an air lock with double doors about 3 ft apart (Fig. 241). This prevents a sudden rush of air when the door is opened. Although good ventilation systems provide air under slight pressure this cannot hold back the large and sudden inflow through an opened door if there is a draught in the room outside.

The method of using the lock is to confirm first that the door to the laboratory is shut and, for this, a small window is needed in the outer one. Then the lock is entered and, after the outer door has been closed again, the laboratory door can be opened.

Sliding doors cause less air disturbance but are difficult to hang without creating dust traps. They cannot be opened easily without using the hands and

this is undesirable because the hands are scrubbed outside the room. Foot operation by niches in, or projections from, the door is not very successful.

Swing doors generally fit better, particularly if their openings are surrounded with insulation strip. They can be made to push open and, therefore, can be foot operated, but footplates are necessary to protect the wood.

A wall hatch between the store and laboratory is useful for transferring equipment into the room before work begins. It should be designed as an air lock so that it can be used during working if necessary.

### E. Surfacing Materials

The floors, walls and bench tops of an asepsis room must be

- (a) Easily cleaned—frequent washing will be necessary to prevent accumulation of dirt
- (b) Smooth—cleaning is easier if there are no cracks and pores in which dust and micro-organisms can lodge
- (c) Impervious, e.g. to cleaning agents and spilt liquids
- (d) Resistant to chemicals. They should not be softened or swollen by solvents, stained permanently by dyes or damaged by strong acids or alkalis

#### 1 FLOORS

The most suitable are

##### (a) Terrazzo

This is a mixture of cement and crushed marble. Both constituents can be coloured. It is spread in plastic form on the site or is obtainable as tiles. In both cases expansion joints of ebonite (black) or P V C (various colours) are essential to prevent cracking. The dust collecting angle at the wall can be eliminated by making a concave sweep with the plastic material or by using concave tiles.

In appearance terrazzo is the most 'aseptic' flooring. It stands up to energetic cleaning and, if desired, the floor can be gently sloped towards a gulley at one side of the room, to carry the water away. On the other hand, it is expensive, cold, tiring to stand on, noisy, slippery when wet, and badly marked by rubber heels. It is attacked by acids and stained by dyes but can be given a protective surface to increase its resistance.

##### (b) Linoleum

Heavy grade linoleum has many good features. It is inexpensive, reasonably warm, comfortable, quiet,

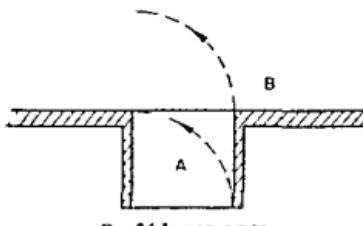


Fig. 241 AIR LOCK  
A—Lock B—Asepsis room

obtainable in many colours and easily cleaned Sheet and tile forms are available but the former is most suitable because a tiled floor has many joints and more surface irregularities to trap dust. It should be sealed to a bituminous base to prevent lifting, displacement and seepage underneath. A plastic coving can be used to cover the wall floor junction. It can be surface-sealed to reduce treading in of dust and marking by rubber heels. The polished surface is slippery when wet and although non slip polishes give some improvement it is safer to restrict treatment to an occasional oiling and frequent damp mopping.

#### (c) Plastics

The non slip or matt finish grades of PVC are suitable for asepsis rooms. They are obtainable as sheet or tiles and the joints can be welded, consequently, the floor can be flooded with cleaning fluids without danger of seepage beneath. The advantages and comments made under linoleum also apply in this case but with the following qualifications—

The polished surface is *very* slippery, and treatment should be limited to washing. Oils and organic solvents attack it and dyes are absorbed. It is less comfortable than heavy grade linoleum possibly because it is thinner. To stand up to trolley wear it should be as thick as possible.

#### 2 WALLS AND CEILINGS

The possible surfaces are

##### (a) Tiles

Although they appear to provide a very smooth, non absorbent, and easily cleaned surface tiles have fallen from favour in recent years. Possibly this is because some older types cracked spontaneously, or became crazed with age or the intermediate cement broke away to leave dust holding cavities. Good quality modern tiles seem more satisfactory and, in particular, the weakest point of this type of surface—the inter tile spaces—can be narrowed because the tiles are lighter and the cements have greater adhesive power.

Ceramic surfaces are cold, encourage condensation and will not stand up to really hard knocks.

##### (b) Hard Gloss Paint on Smooth Plaster

Gloss paint is inexpensive and quite satisfactory when new but must be renewed as soon as cracking or peeling begins. Plaster walls are easily damaged, e.g. if banged with trolleys, and, therefore it is preferable to have tiles or terrazzo to half height.

##### (c) Plastic Laminate Board

This material (see 'Bench Tops') has been used for covering the walls and ceilings of asepsis rooms in industry. The cost is high.

#### 3 BENCH TOPS

The most popular surfaces for asepsis work are—

##### (a) Stainless Steel

This is virtually indestructible. Of the preparations commonly used in aseptic technique, solution of iodine is the only one that noticeably attacks it. The attaching screws should be under the bench not along its edge where they are conspicuous and dirt-collecting.

##### (b) Plastic Laminates

The major advantages of plastics over stainless steel are bright colouring and lower cost. In addition, they are less noisy and not as cold, but they are not entirely satisfactory in either of these respects. Although their heat resistance is good, the radiation from an autoclave over a gas ring can raise and distort the laminate. Resistance to reagents, except strong solutions of phenols, is excellent and although dyes cause staining this is easily removed if treatment is not delayed.

Each bench surface should be made from one complete sheet of laminate because some liquids attack the adhesive at the joints and eventually cause lifting. The bench can be edged with a strip of the same plastic, or with teak or with a U-shaped angle-piece of stainless steel, none of these is entirely satisfactory—the plastic may chip, the teak sometimes separates slightly to produce an undesirable crack and the steel angle gives a dust-collecting edge. (For further information on surfacing materials see Bate (1961) and Guild of Public Pharmacists (1961).)

#### F. Services

An asepsis laboratory will require many, if not all, of the following services—

(a) *Ventilation*. This may include removal of micro-organisms, control of humidity and temperature, and provision of fresh air. It is discussed in a separate section.

(b) *Electricity*, for lighting and, sometimes, for a hot plate, ultraviolet lamp, aerosol producer or vacuum pump.

(c) *Gas*, for the Bunsen burner

(d) *Compressed Air and/or Vacuum*, for clarification and bacterial filtration.

(e) *Nitrogen*, for replacement of air by an inert gas.

(f) Water, not of importance in the laboratory itself but necessary in the washing room  
 (g) A Method of Dealing with Waste

To facilitate dust control, pipes should be hidden either in the wall or, if fixed benches are used, in a totally enclosed space at the back. Fittings designed not to harbour dust should be chosen.

#### 1. ELECTRICITY

Strip lighting is the most pleasant to work in. Dust-collecting surfaces within the room can be avoided by fitting the tubes above flush glass or plastic panels in a false ceiling but it must be possible to get into the ceiling space, or to remove the panels from inside the room, for cleaning the glass and changing the tubes. Switches and sockets should be flush fitting and have finger plates of plastic because metals are less resistant to the fluids used for cleaning and disinfection. Most of the controls can be outside the room, e.g. there is no need for the lighting switch or the ventilation panel to be inside. A red indicator window above the entrance carrying some brief but effective wording, such as 'Keep out', is useful to prevent disturbance.

#### 2 GAS

Gas cocks may be on the wall or at the back of the bench but the controls must be easy to reach. One possibility is a separate bench for each worker arranged end-on to a group of wall-fitted service outlets positioned so that the operator need not get up to use them. However, if staff work facing the wall, the controls should be brought to the front of the bench either at floor level for foot operation (Rugby, 1961) or just under the top at a point convenient for the hand. It is unsatisfactory to have the cock under the bench top because then the tubing comes up from the front and there is a risk that if the burner is near to the edge, as it should be, it will be pulled off by the elasticity of the tubing or by accidentally catching the latter with the arm.

No great advantage is obtained from trying to protect taps and valves from dust by, for example, fixing a shelf immediately above or setting them in a wall cavity. Often this results in dirt passing unnoticed when, in a more conspicuous position, it would not have escaped the cleaner's eye.

#### 3 COMPRESSED AIR AND VACUUM

Some types of rotary pump can separately provide both these services. Pumps are noisy and are best housed outside the laboratory, for example, on an antivibration mounting in a sound proofed cupboard in the washing room. A suitable valve system can be

included in the pipeline near to the pump and set to give either vacuum or air when the pump is switched on. Supply at the benches is most suitably controlled by needle valves.

If the pump cannot provide air this may be supplied to a separate pipeline system from a cylinder.

#### 4 NITROGEN

A cylinder can be kept near to the vacuum pump and connected via a reducing valve and flexible hose to another pipeline leading to a second needle valve at the bench.

#### 5 WATER

It is difficult to justify a water supply in the laboratory itself. Hand-washing facilities are undesirable because of the risk of splashing organisms from the hands into the air and on to working surfaces or equipment. Occasionally it is necessary to cool a sterile cream quickly by shaking the jar under a stream of cold water but this can be done by someone outside the room, by passing the preparation through the hatch. Water baths, sometimes needed to melt or soften semi solid bases or to heat anaerobic culture media, can be filled in the washing room before work starts, they are rarely used long enough for a constant level type, which would need a water supply, to be an advantage.

In a hospital washing room two or three sinks may be enough but in industry much larger facilities are essential. Stands of the drinking fountain type are economical with space because at least four workers can use each at one time. Pressure on a foot control causes water to spray down into a large circular trough. An alternative is a method popular in operating theatre suites, elbow-operated taps are arranged along tiled walls and the water falls into a gulley, the users being protected from splash by adjustable sheets of armoured glass at waist level (Fig. 24.2). Individual pedestals with knee-operated taps are obtainable. In all types there is no need to use the cleaned hands for turning off the water. A comfortable temperature is produced by fitting mixing valves. Soap is conveniently supplied, in liquid form, from dispensers on the wall or on the tops of fountains. Electric hand driers are often very large, are usually slow and cause considerable air disturbance, paper towels, sterile if preferred, are an acceptable alternative.

In some industrial suites showers are provided, but this extreme precaution is justified only if other measures are equally stringent, for example, a complete set of clean clothes must be put on afterwards.

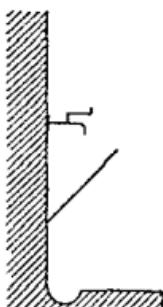


Fig. 24.2 UNIT FOR SCRUBBING-UP

#### 6 WASTE DISPOSAL

Wrapping paper, bags, plugs, pieces of twine, elastic bands, tops of ampoules etc collect during aseptic technique and must be cleared from the working surface immediately. A foot-operated waste bin is popular but unless the bench is very low it is not possible to reach the pedal without partly sliding from the chair, also the mouth is rarely large enough to receive large pieces of paper. A better method is to fit, at the side of the knee-space, a metal ring that can be swivelled under or out from the bench and into which can be slipped a plastic or stainless bucket or large bowl, this can be close enough to the hands to make effective and, as is highly desirable, subconscious use, possible.

#### Furniture

##### 1 BENCHES

Drawers and cupboards are not essential if the laboratory is used solely for manipulations. Therefore, to reduce dust collection and facilitate its detection and removal, conventional benching may be replaced by tables or wall-mounted work shelves. With these the only place where dirt is likely to be overlooked is the undersurface of the top and this must have regular attention. For movable tables, wall service points are necessary.

When storage space is considered essential, cupboards are preferable to drawers because the latter are less easy to clean and are more likely to be left slightly open. The cupboards should be dustproof, e.g. the doors can close against flanges to prevent dust passing straight through the cracks at the edges, excellent protection is obtained if these flanges are covered with foamed plastic draught excluder. An acceptable way of including drawers is to fit a nest inside a dust proof cupboard. To assist cleaning,

as many surfaces as cost permits should be faced with plastic laminate, e.g. the fronts of cupboards and drawers and, possibly, the entire insides of both. Rounded beading may be used to cover the dust-retaining angles within storage spaces and between fixed benches and the walls. Sliding doors are more difficult than hinged types to make dustproof and to clean, in particular, their runners are avid dust-collectors.

Small units on castors make useful additions to the working space. They can be made low enough to slide under the bench when work has finished, and cupboards and drawers can be included if required. One at the side of each worker provides, within easy reach, a place for unused and used equipment (Fig 24.3). The castors must be braked to prevent unexpected and, possibly, dangerous movements during use.

A suitable size of working surface, excluding any additional unit, is approximately 4 ft x 2 ft. Height is not critical since aseptic technique is carried out seated, and adjustable chairs are used. Adequate knee space must be allowed.

##### 2. SEATS

These must be adjustable and comfortable. The former of these two features ensures that the user's face is well above the front opening of the screen and, therefore, that breath is kept away from the materials underneath.

Discomfort is likely to distract attention and cause unnecessary and inefficient movements. Comfort is best assured by chairs rather than stools and by upholstered, rather than wood or metal, seats and backs. The fabric must be washable. Unfortunately, in general, upholstered, adjustable,

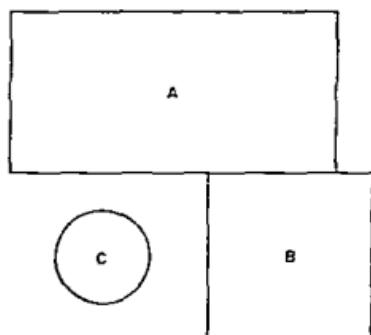


Fig. 24.3  
A—Working space B—Leg C—Seat

chairs have an undesirable number of dust retaining crevices

### 3 TROLLEYS

Trolleys with removable trays, preferably of stainless steel, are better than tables, benches or cupboards for holding spare equipment. They are easy to clean, can be taken away for replenishment and, if necessary, the trays can be steam sterilised.

### 4 DOORMAT

The soles of outdoor shoes are heavily contaminated with dustborne organisms and, unless special footwear is worn in the laboratory, it is useful to have, in the air lock, a mat part immersed in a detergent-disinfectant solution. It can be contained in a floor-well of terrazzo or in a shallow tray.

### 5 SCREENS

Usually, aseptic technique is carried out under a screen. There are two basic designs—

#### (a) *Shack Types* (Fig 244)

Originally these consisted of a wooden case with a sloping front of glass, but now, the most common fabrication material is 'Perspex'. Plastic screens are transparent, giving excellent visibility of the contents, and light in weight, which makes them easy to move about. By using piano hinges, they can be made to fold, for storage in a narrow space after use; dust is excluded at the hinges by fitting a layer of strong, flexible, transparent plastic underneath. Plastic accessories, such as a holder for a distribution apparatus and a connexion for tubing to air or vacuum can be attached easily. The sloping front should raise for the introduction of large items of equipment such as a balance.

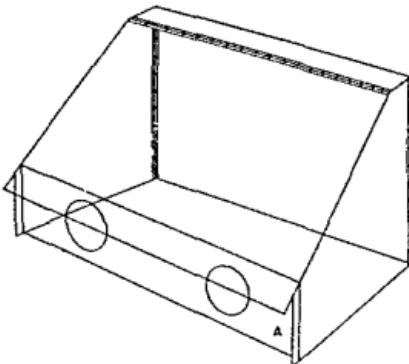


Fig 244 PLASTIC FOLDING SHACK TYPE SCREEN

If clean air is supplied to the room, there is no need to enclose the screen front and work through arm holes or rubber sleeves, which restrict natural movements and lead to far less smooth technique and, sometimes, clumsiness. An efficient operator can maintain excellent asepsis in an open fronted screen even in a room without a special air supply.

#### (b) *Fume cupboard Types*

Tall apparatus, such as burettes, has to project through a hole in the screen top when used with the shack type. Fume cupboard types are high enough for this equipment to fit entirely inside. Early designs were of wood and glass and had a front that lifted on sash cords like a chemical fume cupboard but folding plastic types in which part of the front is hinged to lift up and admit the apparatus, have been developed.

## CLEANING METHODS

An asepsis laboratory must be cleaned often enough to ensure that dirt can never be found.

### Dry Cleaning

#### FLOORS

Brooms are unsuitable because they disperse large amounts of dust into the air. Vacuum cleaners are very satisfactory if the bag has a paper insert to prevent tiny particles from passing through the fabric. Bate (1961) has discussed other desirable features of hospital vacuum cleaners. Mops impregnated with dust retaining oil are useful but need regular cleaning and re-impregnation.

A method of dust laying that can be used for semi-porous surfaces, such as unpolished linoleum, is occasional treatment with spindle oil. This should not be applied too liberally and the surface needs regular washing to remove retained dust.

### Wet Cleaning

#### FLOORS

Mopping with really hot water containing detergent is adequate if the mop is washed daily and, during use, is squeezed into a second bucket to minimise the transfer of dirt from one area to another.

**WALLS AND FURNITURE**

Wiping with a slightly damp, clean cloth is sufficient. Upper walls and ceilings cannot be treated as often but regular damp mopping is practicable in small laboratories

**Disinfection**

Frequent disinfection is unnecessary in a well ventilated and efficiently cleaned room.

The wet technique, described above, can be used for the floor but the mop should be soaked the previous night in a separate quantity of the solution, to saturate it with the disinfectant and thus prevent reduction of the strength of the working solution to below effective level. This is necessary because disinfectants are sometimes strongly adsorbed on to

fibres. Alternatively, the surface may be flooded with the solution and, later, sucked up by a wet vacuum cleaner. In both cases the disinfectant should be left on the floor for several minutes before removal.

Among the classes of germicide recommended for floors and surfaces are synthetic phenols, quaternary ammonium compounds and iodophors (Bate, 1961, Walter and Errera, 1960). Inexpensive, household disinfectants have powerful smells that persist long after use. Recent, but insufficiently proven developments include germicidal floor polishes and mop-impregnating oils, and disinfectants with persistent activity on surfaces.

Ideally, cleaning should be done at the end of the day to give plenty of time for the resulting air disturbance to subside before the room is used again.

**CLOTHING**

Special clothing for the staff of asepsis rooms has been studied most thoroughly in industry (e.g. Jones, 1952) where factors such as the complexity of the processes and the large number of operatives in the area necessitate precautions that are less important when simpler techniques are carried out by one person in a hospital. Consequently, the subject will be discussed from the industrial viewpoint first and, later, features that might be relaxed in less exacting circumstances will be indicated.

In deciding how much clothing was essential, and the design of each item, Jones took the following factors into account—

- 1 Maintenance of the highest standard of asepsis
- 2 Comfort and appearance—since either can affect the quality of work
- 3 Ease of changing—because the impatience caused by a complicated regimen may lead to neglect of important features

In theory, to obtain maximum protection the body must be covered completely since micro-organisms may be shed from the skin, hair, respiratory tract and normal clothing. In practice it is usually possible, in the interests of comfort, appearance and convenience, to modify this requirement in several respects without seriously affecting the standard of asepsis.

**GOWNS AND TROUSERS**

A gown is the most essential item. It must completely cover the clothes underneath, even when the wearer is seated. Therefore, it must be long e.g. to mid-calf. Suitable designs include the surgeon's

gown, which has no opening at the front and is fastened by tapes or buttons at the back, and the double-breasted overall coat that buttons up to the neck and has a stand up collar. Buttons should be of rubber, to withstand sterilisation. Long sleeves should fit snugly at the wrist (e.g. see Brewer, 1948) unless rubber gloves are worn, when loose material can be folded into the glove cuff.

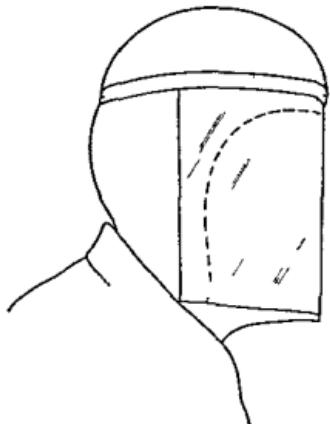
Appearance is particularly important in gowns for women. Excessive fullness is uncomplimentary and is unsatisfactory from the viewpoint of good asepsis because the loose material flaps and produces air movements.

Long trousers are not often worn. The advantages appear to be slight and the additional changing requires extra time and, possibly, privacy.

Usually gowns are made from cotton and linen because these fabrics can be steam-sterilised. They are not ideal because movements shed tiny fibres into the atmosphere and protection from contamination from the underlying skin and clothes is incomplete, especially if they get wet. Gowns of waterproof plastic would solve both problems but might be difficult to sterilise.

**HEAD DRESS**

The hair, particularly if long and loose is very likely to scatter dust and skin fragments. Complete enclosure in a hood, long enough to tuck into the neck of the gown, is desirable (Fig. 24.5). This type of head-dress is quite elegant and is difficult to put on incorrectly. A cap into which the hair is pushed is much less decorous and, therefore, the temptation to leave out some of the hair is great.



*Fig 24.5 HEAD DRESS AND PLASTIC FACE MASK*

#### MASKS

Covering the nose and mouth will not necessarily protect the working area from micro-organisms of the respiratory tract. The efficiency of a mask depends on the material, design, fit and period of use.

Gauze, muslin, linen, card paper and transparent plastics are among the many materials that have been investigated. All allow a large number of the particles from a cough or sneeze to escape through the mask or at its edges. Although plastic, card and some paper types are impervious to bacteria they usually fit the face less well than fabrics, and the overall escape of organisms is often greater. Some of the best results have been obtained with masks made from two layers of gauze of 80 threads/in or six layers of 42 threads/in—the first of these had a soft metal strip, that could be moulded to fit the bridge of the nose, sewn into the top. Well-designed masks not only fit the cheeks and nose well but are, also, a good fit under the chin; in asepsis this is particularly important because downwardly-directed air is more likely to enter the screen.

Masks should be sterile and changed frequently. In use, droplets dry on or in the material and some of the nuclei become entrained in the expired air which may eventually contain more bacteria than the unmasked breath. Walter and Errera (1960) recommend a change every half hour. The rubbing

of masks against the skin can cause particles to separate and, to reduce this, the use of face cream has been suggested.

Jones (1952) describes a mask used in penicillin filling rooms. It is fitted over a hood and consists of a curved sheet of plastic attached to a ring that fits round the head. Linen connected at top and bottom gives virtually complete enclosure of the face. As the plastic does not touch the skin, there is no discomfort and, therefore, no temptation to touch the mask and thus contaminate the fingers. The face cannot be scratched and if skin fragments separate they cannot easily escape into the atmosphere. A head-covering of this kind (Fig. 24.5) protects the skin and eyes from ultra violet radiation when this is used to reduce the microbial content of the air.

#### GLOVES

The wearing of sterile rubber gloves for aseptic work presents more complications than advantages. Some articles that are externally unsterile must be handled, and gloves become contaminated quickly. Unqualified staff are apt to ignore this and to continue touching sterile surfaces and, even, substances. In these circumstances the risk of contamination is increased instead of decreased. Also gloves are costly, difficult to sterilise, short lived and uncomfortable.

It is better to use a non touch technique instead. In this the hands are considered to be bacteriologically dirty, although they have been washed and disinfected. On the rare occasions when a sterile article must be touched, short sterile finger stalls or even squares of rubber can be used, e.g. for holding a skirted rubber cap steady while it is being put into the bottle.

Severe dermatitis and other allergic reactions are developed by some people if they come into contact with streptomycin or penicillin solutions. When, in aseptic technique, there is danger of contaminating the skin with these antibiotics it is advisable to wear gloves but the non touch technique should still be practised.

#### FOOTWEAR

Because of the load of infection on outdoor shoes it is advantageous to change them. Tennis and rubber shoes and surgeon's boots are uncomfortable if worn for long. Leather shoes with low heels and rubber soles are satisfactory and easy to keep scrupulously clean if never used outside the suite.

#### ASEPSIS ROOM VENTILATION

The most important function of the ventilation system of an asepsis room is to provide and maintain

a low level of macro-organisms in the air but, if cost allows, it can also be used to control the

temperature and humidity. The design of a suitable installation is the province of a ventilation engineer with specialised knowledge of the problems of supplying bacteriologically clean air. The following account attempts no more than a background to the subject.

The processes involved in a typical ventilation system are, in order—

- 1 Intake of fresh air
- 2 (Sometimes), admixture with air exhausted from the room.
- 3 Cooling, if necessary
- 4 Dehumidification or humidification, if necessary
- 5 Heating, if necessary
- 6 Removal of micro-organisms
- 7 Admission to the room at a rate that will maintain a satisfactory temperature and relative humidity and, particularly, a low level of bacterial contamination, even when work is in progress

### **1. Intake of Fresh Air**

Filtration is the most popular method of removing airborne organisms. Rapid clogging of filters by dust, with consequent reduction in rate of air flow and need for early replacement, can be prevented by siting the intake high above the ground and away from chimneys. This position excludes most of the pathogen-contaminated dust particles found at street level. The duct entrance should be easily accessible for regular cleaning and guarded from nesting birds and inquisitive cats. The main filter is usually further protected by a coarse prefilter which removes larger dust particles.

### **2. Admixture with Exhausted Air**

Often the fresh air is mixed with air extracted from the room, the recirculated portion being about 75 per cent of the whole. The main advantage is that less heat is necessary to warm the air in winter but, if the plant has equipment for humidity control, the saving may be less than expected because dehumidification causes cooling and, therefore, necessitates reheating.

### **3. Temperature Control**

An advantage of heating an asepsis room by the ventilation system is the elimination of radiators. These are dust collectors and distributors and even when set in the wall and fronted with a flush panel still manage to distribute dirt at the edges. They also cause air movements that can disturb the carefully designed downward flow from a displacement-type ventilation system (*q.v.*)

The heater section of a ventilation plant usually warms the air by pipes containing hot water or steam and is thermostatically controlled to give an air temperature of about 65° to 70°F.

Cooling is rarely necessary, except, perhaps, in some teaching laboratories in Schools of Pharmacy where failure to turn down burners when they are not in use often produces an uncomfortable temperature rise. Coolers are similar in design to heating units but a refrigerant is circulated through the tubes.

### **4. Humidity Control**

The atmosphere of an asepsis room may become unpleasantly dry if several Bunsens are burning and/or the heating unit is operating. To increase the humidity to a comfortable level of about 55 per cent a humidifying plant is required. In this the air is passed through a fine atomised spray of fresh mains water. Some humidifiers recirculate water from a storage tank and, because of the danger of microbial growth in the latter, a germicide should be added when this method is used.

High humidity is not often a problem except in the industrial asepsis rooms in which hygroscopic substances such as penicillin are filled into vials. Jones (1952) recommends 45 per cent for penicillin and points out that lower humidities are uncomfortable and cause the accumulation of static charges on the powder which interfere with free flow. Usually humidity is lowered by condensing the excess vapour in the cooling unit but it has been effected by completely drying part of the air with silica gel and then mixing it, in suitable proportions, with normal air.

### **5. Provision of Bacteriologically Clean Air**

In the pharmaceutical industry, bacteriologically clean air is required for two major purposes—

(a) For the aeration of those fermentation processes in which aerobic organisms are required to grow in submerged culture, i.e. throughout a deep tank of medium, in the lower part of which the oxygen concentration would be insufficient without the supply of additional air.

(b) To produce satisfactory atmospheres for aseptic dispensing and filling, and sterility testing.

Fermentation air must be sterile because it comes into intimate contact with the material (the culture medium) that requires protection. As this medium has been formulated to provide an ideal environment for the production organism it usually encourages the vigorous multiplication of contaminants as well. The resulting competition for nutrients, liberation of

inhibitory metabolites and destruction of the fermentation product can cause drastic reduction of yields. The outstanding example is the breakdown of penicillin by penicillinase producers.

In asepsis rooms air that is not sterile can be accepted provided it has a high degree of bacteriological purity. This is because a major aim of aseptic technique is to prevent contact between room air and the sterile materials being processed. Therefore, when a skilled operator is working, if any contacts occur they are so brief that contamination is unlikely.

### Methods

Bacteriologically clean air can be produced by removing the micro-organisms by mechanical filtration or electrostatic precipitation, or by destroying them with heat, ultra-violet light or chemicals.

Heat is too costly for small laboratories. Chemical methods are slow and unreliable and cannot be used for fermentation air because the bactericidal agent might be carried over into the fermenters. Ultra-violet light has poor penetrating power and, therefore, little effect on bacteria in dust particles. The capital cost of electrostatic precipitators is high. Filtration is the most suitable method for small rooms and is often preferred for large installations, including fermentation plant, because it is efficient, simple and relatively cheap.

## I MECHANICAL FILTRATION

### A Mechanisms

A filter in which the pores are smaller than the bacteria is the only type that would be expected to have an efficiency of 100 per cent. However, filters that strain out organisms in this way choke very quickly because almost all the organisms are collected on the upstream surface, in addition, the smallness of the total pore area causes high resistance to air flow.

Fortunately, straining is not the main way in which small particles are removed from air by an efficient filter. The majority are retained on the surfaces of the fibres by intermolecular forces and, in some media, by electrostatic attraction. These mechanisms operate throughout the thickness of the filter and, therefore, a much larger collecting area is available for filtration than if only the upstream face was used. For this reason, filters with pores much larger than bacteria can remove micro-organisms efficiently. Successive dislodgement and entrainment of the particles might be expected to carry them gradually through the filter but generally this does not happen (see, for example, Terjesen and Cherry, 1947).

Consequently, the methods used for purifying the air of a small asepsis laboratory often differ in nature or degree from those used in the fermentation industry. (Small laboratories are specifically mentioned because the large asepsis filling rooms of industry are often supplied with air of fermentation quality.) Although this chapter is concerned primarily with aseptic technique in relation to the preparation of injections it is simpler to discuss the provision of air for asepsis rooms and fermenters together. This has been done in the following sections—

To obtain maximum advantage from such a retention mechanism, optimum conditions must be provided for collisions between the particles and the filter but this is complicated because at different velocities different forces are responsible for the contacts.

At low velocities a diffusion mechanism predominates. Brownian movement makes particles knock against the filter and their retention reduces the concentration in the air adjacent to the fibres, consequently, more particles diffuse into this region from the passing dust cloud. Since Brownian movement is a characteristic of small particles this mechanism is most important for fine dusts, ( $1\text{ }\mu$  and less).

As the air velocity is increased the diffusion mechanism becomes less important and is gradually replaced by an impingement mechanism which will be explained by reference to Fig. 24.6. *A* represents the cross section of a fibre in the filter bed, the lines show the streamlines and the arrows the direction of the air flow. The only particles that are certain to be stopped are those in the central streamline, and these are said to be directly intercepted. The particles in the other streamlines will be carried around the fibre unless they can acquire sufficient momentum to maintain a straight course and, therefore, impinge on the fibre. Since the momentum of a particle is the product of its mass and velocity the probability of impingement is raised if the airstream velocity is high and the particle size is large. Consequently, as the velocity increases more and more particles acquire sufficient momentum to impinge.

The decrease in efficiency of diffusion with rise in velocity is not immediately replaced by a corresponding increase in impingement efficiency, therefore, there is an intermediate velocity, for each size of particle, at which the efficiency of a filter is at a minimum. This effect is most significant for small particles (Stairmand, 1950).

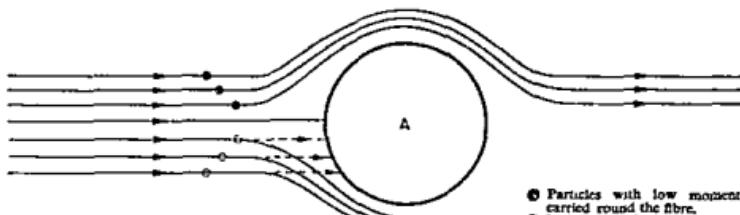


Fig. 246

- Particles with low momentum—these are carried round the fibre.
- Particles with high momentum—these maintain a straight course and impinge on the fibre.

The significance of the various factors in practice is as follows

**Straining.** Generally, this is only important for very large particles because the interstices of many filtering media are large

**Direct interception** Only in the velocity range of minimum efficiency is this the major factor

**Diffusion** If very low velocities are used to obtain maximum advantage from this mechanism a considerable filter area is required to provide sufficient air to the room or plant. To some extent this can be achieved by corrugating the medium, but often an uneconomically large bank of filters is necessary

**Impingement** Since, for the reason just given, flow rates optimal for diffusion may be impracticable, higher velocities, at which impingement predominates are often used. In the production of bacteriologically clean air it is desirable to avoid the velocities at which the total collection efficiency for small particles is low, these range from 0.1 to 2 ft/sec, approximately (Stairmand)

**Electrostatic Attraction** This is important in some media, e.g. glass and asbestos (see 'Liquid filtration') It can be induced in animal and vegetable fibres by coating with suitable resins

(For further information see Davies (1952) and Humphrey and Gaden (1955))

#### B. Types of Filter

The choice of filter for fermenter air is limited because of the high efficiency (virtually 100%) that is necessary, but for an asepsis room, where the standard of purity acceptable to the user may vary, the selection is wider. Filters can be divided, roughly, into three main groups—

##### 1. COARSE FILTERS

If experience shows that a concentration of one or two bacteria carrying particles/ $\text{ft}^3$  does not constitute a hazard to aseptic processing, as might be the case for simple operations in a small room, then one

of the more retentive types of filter used for general air conditioning may be adequate. An example is the Vokes 'Kompak' K600 which removes 99.9 per cent of dust particles down to  $5 \mu$  and has been successfully used for operating theatres by Blowers and Crew (1960)

#### 2 HIGH-EFFICIENCY FILTERS

When a higher standard is desirable as, for example, in large rooms accommodating many workers, without screens, more efficient filters are available. These are classified by the Methylene Blue Test (q.v.) in which the percentage penetration of methylene blue dust is found. For a high-efficiency filter this would not be more than about 35 per cent and, since the dye particles are very small, ranging from approximately  $1.3 \mu$  down to virtually zero, very few micro-organisms will escape removal. The Vokes Composite 'Kompak' K600 is an example

#### 3 ABSOLUTE FILTERS

The need, in the fields of nuclear energy, for filters to retain the finest particles of radioactive dust has led to the development of even more efficient media with methylene blue penetrations of less than 0.1 per cent. These can be used to provide air of superlative quality to asepsis rooms if the advantage is considered to justify the cost. Examples are Vokes cellulose-asbestos or glass filter papers which have methylene blue penetrations of less than 0.05 per cent

In addition to their higher purchase and replacement costs, finer filters have smaller air capacities and it may be necessary to install more than one, or to increase the size of a bank, to provide the required rate of air-flow

#### C. Filter Beds

Filter beds may consist of granules, loosely packed fibres or filter papers

## I GRANULES

This type of bed has been used in the antibiotic industry. The most successful material was activated charcoal. Air was passed up a tower containing the granules, which were prevented from lifting by a fixed screen over the upper surface. The granule size was varied according to the depth of the filter and ranged from 10 mesh for deep beds to 60 mesh for shallow ones. Adsorption was believed to play an important part in particle retention.

The method lost favour when it was found less reliable for fermentations in which the initial pH of the medium was nearly neutral. Most of the early work had been done on the penicillin fermentation where the medium was initially acid (pH 5.5 ca) and, therefore, inhibitory to most of the occasional contaminants that passed through the bed. On the other hand, the Streptomycin fermentation, which is started at a pH of 7.35, is at the optimum for bacterial growth and contaminants grow readily.

(For further information see Hastings, 1954)

## 2 PADS OF LOOSELY PACKED FIBRES

These may be animal, vegetable or mineral and except for some slag-wool types are loosely packed. There are two main types of unit. In one the fibres are enclosed in a flat, square or rectangular, casing which fits so snugly into a holder in the ventilation trunking that by passing at the edges is impossible. In the other, the medium, in the form of long sheets (laps) is pleated around a hinged wire frame (Fig. 247) in order to expose a large surface to the air in the minimum space (e.g. in the Vokes 'Kompak' K600 the filtration area is 13.5 times the frontal area), the frame also fits tightly into the ducting.

### (a) Animal Fibres

One example is merino wool treated with resin to increase electrostatic attraction but this type of medium is relatively uncommon.

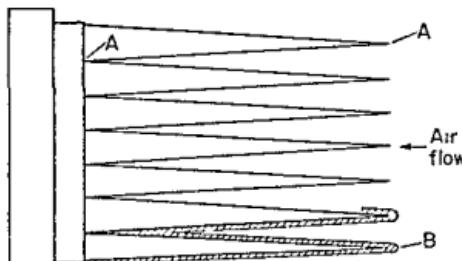


Fig. 247 PLEATED LAP FILTER (EDGE VIEW)  
A—Hinges B—Lap

### (b) Vegetable Fibres

*Cotton Wool* The use of non absorbent cotton wool for air filtration naturally followed its success as a closure for containers of sterile liquids in bacteriology and aseptic technique.

Carter (1961) found that at a flow rate of 80 ft/min bacteria were entirely removed from air by a depth of at least 8 in packed to a density of 8 lb/ft<sup>3</sup>. However, when a few bacteria per ft<sup>3</sup> are not considered a serious hazard it can be used in thinner layers, e.g. in the Vokes Multivec unit a thin layer of cotton wool is attached to a metal gauze which has V-shaped corrugations to increase the filtration area. This unit was found by Sykes and Carter (1953) to be 98 to 99 per cent efficient at removing spore infected dusts when combined with a glass-fibre prefilter.

The disadvantages of cotton wool are—

- (i) It becomes clogged with dust quickly and although cleaning is possible in some cases (e.g. the Multivec can be vacuum-cleaned several times) frequent replacement is necessary.
- (ii) Its mechanical strength is poor and therefore, the air flow tends to pack it more tightly, with consequent increase in resistance.
- (iii) It is wetted more easily by damp air and steam sterilisation than many other materials. If this occurs badly and the filter is not dried before use, bacteria may swim in moisture films on the fibres, or be entrained in droplets, from the dirty to the clean side of the filter.

### (c) Mineral Fibres

*(i) Slag Wool* Slag is a vitreous residue from the smelting of ores. It is disintegrated into fibres by allowing a molten stream to drop into a horizontal blast of steam (de Dani, 1955).

It has been used very successfully for providing fermenter air but the resistance to air flow of the compressed blocks (the form most often used) is higher than is generally desirable for room ventilation.

Its fibres are small (under 10 µ in diameter) but very strong and, unlike cotton wool, the pad is not compacted or disarranged by the air flow.

Terjesen and Cherry (1947) and Cherry, McCann and Parker (1951) fully investigated this type of medium and recommended use of a small fibre size (majority less than 6 µ in diameter) and a low air velocity, both of which favour collection by diffusion. They found that organisms penetrated to about 2 in and, therefore, suggested a minimum of 3 in. They also emphasised the importance of an adequate

and, particularly, a uniform packing density. If the density is uneven, air velocity and, therefore, efficiency differ in different parts of the filter.

Repeated steam sterilisation reduces its efficiency by creating channels through which organisms can pass. Dry heat at 170°C for 2 hr has been used, one method of achieving this is to put heaters in the upstream trunking and adjust the air flow to keep the filter at the correct temperature.

Small fibres tend to separate from the sterile, downstream side of the pad and where these are objectionable, e.g. in the atmosphere of an asepsis room in which parenteral preparations are made, an additional filter should be fitted to retain them.

If a prefilter is used to prevent clogging of the comparatively fine surface pores, slag wool filters remain effective for at least a year.

(ii) Glass An interesting account of the manufacture and applications of glass fibres is given by de Dani (1955) and the formation of filter mats from these fibres is described by Wente and Lucas (1956).

Glass fibre pads are commonly one or two inches thick. One popular type has a fibre diameter range of 50 to 200  $\mu$  and a packing density of 10 to 20 G/ft<sup>3</sup> (cf. slag wool—diameter mostly 6  $\mu$ , packing density not less than 17 lb/ft<sup>3</sup>), the smaller fibres are on the clean side and are more closely packed. As this is a coarse filter, impingement is the chief retention mechanism, and high velocities can be used to take full advantage of this because the open structure prevents large back pressures. The fibres are treated with an adhesive. Carter (1961) reported that at a flow rate of 5 ft/sec the efficiency was rather low, ranging from 80 to 90 per cent for normal airborne contamination, but increase in flow rate produced considerable improvement. This grade is very suitable as a prefilter. Pads with much smaller fibres, 1 to 2  $\mu$  diameter and a higher packing density are obtainable, their methylene blue penetration is about 0.5 per cent. The higher efficiency of Vokes Composite 'Kompak' compared with the Kompak is due to a backing of fine glass-wool.

Glass, like other mineral filter fibres, but unlike fibres of vegetable and animal origin, is non-inflammable, non-hygroscopic, and not attractive to vermin. Some types can be dry heat sterilised.

(iii) Asbestos This must be blended with cotton or wool because, when used alone, it compacts badly and, therefore, offers high resistance to air flow. Sometimes, it is incorporated with other materials in a pad or lap because it electrostatically attracts particles of charge opposite to itself (see 'Liquid Filtration'). A 1-in pleated lap containing equal amounts of asbestos and cotton wool, com-

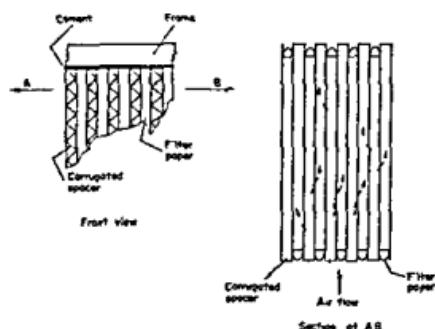


Fig. 24.8 DETAILS OF AN ABSOLUTE FILTER-PAPER FILTER

(Courtesy Vokes Ltd.)

pressed to a packing density of 45 G/ft<sup>2</sup> has been reported to have a methylene blue penetration of only 0.005 to 0.001 per cent at an air velocity of 0.05 ft/sec (Smith and White, 1962).

### 3 FILTER PAPERS

Compressed papers are a comparatively recent development in air filtration media.

#### (a) Cellulose-asbestos

The cellulose (from esparto grass) and asbestos fibres are compressed into a sheet only about 0.02 in. thick. A continuous length of this is folded around corrugated spacers and then cemented into a casing (Fig. 24.8). A case of 2 ft  $\times$  2 ft  $\times$  1 ft may contain a filtration area of 250 ft<sup>2</sup>. At an air velocity of 0.05 ft/sec the maximum methylene blue penetration is 0.05 per cent, i.e. this is an absolute filter.

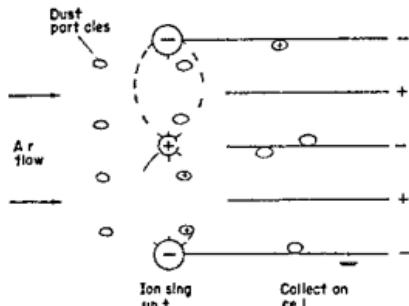
#### (b) Glass

Fine glass fibres of 1 to 2  $\mu$  in diameter are compressed and the sheet is mounted as above except that the spacers are of expanded aluminium to make the whole unit non-inflammable. They are unaffected by humidities up to 100 per cent, for cellulose papers the limit is 80 per cent. The methylene blue penetration may be as low as 0.01 per cent.

The autoclave air filter, mentioned in chapter 22, contains three layers of very fine fibreglass lap as a prefilter, followed by a glass paper. The methylene blue penetration of this is 0.002 per cent. Glass fibre papers are also used in the antibiotic industry.

## II ELECTROSTATIC PRECIPITATION

An electrostatic precipitator is essentially a type of filter because it removes solid matter from the air



**Fig. 24.9 PRINCIPLES OF THE PRECIPITRON ELECTROSTATIC PRECIPITATOR**  
(Courtesy Sturtevant Engineering Co. Ltd.)

but, unlike mechanical filters it is not less efficient at removing small particles

The Precipitron is an example. The air first passes through an ionising unit containing alternately spaced, earthed tubes and thin wires. The wires are charged to about 13 000 V d.c. and the resulting high potential difference between these and the tubes sets up a powerful electrostatic field. This increases the normal movements of the air molecules causing more frequent and intense collisions which, in turn, bring about ionisation. The positive ions travel at great speed from the wires to the tubes. As a dust particle passes through the unit some of the ions become attached to its surface, making it positively charged (Fig. 24.9).

The air then passes into a collection cell containing a series of flat parallel plates alternate members of which are earthed while the others are positively charged to about 6 000 V. The voltage gradient between each pair of plates drives the charged dust particles on to the earthed plates where they adhere. At intervals the collector cell is washed with water.

Efficiencies of 95 to 97 per cent for the removal of bacteria have been reported by Munden (1952) but although better results are possible with other types of precipitator, Carter (1961) was unable to find a record of any experiment in which 100 per cent efficiency was shown consistently.

Although the initial cost is high, the method is an attractive alternative to mechanical filtration for aseptic rooms unless a very high standard of bacteriological purity is required.

### III HEAT

Heat was shown to be a feasible method of air sterilisation by Bourdillon, Lidwell and Raymond (1948). They estimated that all organisms would be

destroyed instantaneously at about 300°C. This has been confirmed by Elsworth, Telling and Ford (1955) who concluded that at 300°C less than one spore in 470 million would survive treatments of 0.14 and 1.6 seconds in electric sterilisers of 140 and 1,700 litres/min capacity respectively.

A short exposure is essential because it is impossible to hold large volumes of air, which for fermentations may exceed a million ft<sup>3</sup>/hr, in a sterilising chamber.

Although this is the most certain method of air sterilisation, the operating costs are high and, therefore, at present, its use is restricted to small volume applications in the fermentation industry, such as experimental work up to pilot scale and the aeration of seed tanks on the plant.

### IV ULTRA-VIOLET LIGHT

The use of ultra violet light as a sterilising agent is discussed in chapter 28. The main points relevant to its use for purifying atmospheres are—

(a) Its penetrating power is poor and, therefore, it does not reach organisms inside dust particles and large droplets. It should be regarded solely as a means of destroying bacteria that are naked or within tiny particles and, therefore combined with another method, such as filtration, that can deal successfully with large particle infection.

(b) Continuous irradiation of the atmosphere is desirable and therefore, workers must be protected from the harmful effects of the radiation on skin and eyes.

(c) It is less effective at low humidities.

(d) Installation and replacement costs are heavy but the method is clean and the equipment easy to maintain.

A lamp inside a screen is a useful aid to aseptic processing.

### V CHEMICALS

For many years there has been considerable interest in the killing of air borne pathogens by methods suitable for occupied public buildings such as schools, offices, factories and places of entertainment. Filtration, electrostatic precipitation and heat require costly mechanical ventilation systems and, moreover, since the aim is to prevent cross infection it is better to use a method that begins to act on the organisms as soon as they are liberated from the respiratory tract and clothes. Therefore, investigators have concentrated on ultra violet radiation and chemical sprays or vapours. From their work on chemicals substances have been discovered and

techniques developed that are suitable for use in asepsis rooms when better methods are not available

A good aerial bactericide should be—

- (a) Rapidly bactericidal in low concentrations even when the micro-organisms are protected by dust and dried particles
- (b) Effective at normal temperatures and relative humidities
- (c) Easily dispersed into and persistent in the air
- (d) Harmless to occupants, including freedom from irritancy to the respiratory tract and eyes
- (e) Non wetting
- (f) Invisible, inodorous, non inflammable and non corrosive to fittings

#### A. Mode of Action

The efficiency of an aerial bactericide is influenced by its physical condition in the air, i.e. whether it is present as a mist or a vapour. A mist might be expected to be more effective because the tiny droplets will wet the bacteria-carrying particles, many of which will be dry or partly dried, and provide moisture to aid penetration of the bactericide and potentiate its killing mechanism. However, the high killing rates obtained with some substances cannot be explained on the assumption that contact between a bacteria-carrying particle and a mist droplet is a necessary preliminary to bactericidal action because it has been shown theoretically that the number of droplets required is far greater than the number actually present during the disinfection process. On the other hand, if sprayed droplets of bactericide are assumed to evaporate almost immediately, the number of vapour molecules produced is more than enough to ensure the required number of contacts. Therefore, it is assumed that many, but not necessarily all, bactericides act through the vapour phase. This is confirmed by the efficiency of some compounds when they are vapourised directly into the air. Lidwell (1948) has pictured the action of an aerial bactericide in the following way—

(a) Vapour molecules of the germicide diffuse through the atmosphere to the particles at a rate determined by the Langmuir formula (Cooper and Gunn, 1957)

(b) The molecules condense on the surface of the particles where they dissolve in the water present

(c) The bactericide diffuses into the particle and apparent death of the associated organisms results if a sufficiently high concentration is reached to inhibit their growth when the particle is collected on a culture medium

The vapour theory also helps to explain several aspects of air disinfection—

(a) Substances with a low vapour pressure are often more effective than compounds with a high vapour pressure. The former condense more readily because of their low solubility in air.

(b) Usually, aerial bactericides are less effective at low humidities (60 to 70 per cent is the optimum for most compounds). At low humidities the bacteria-carrying particles lose moisture, this reduces their size and, therefore, the surface on which condensation can occur. Also, the rate of diffusion of the bactericide into the particle will be slower.

(c) Glycols are more effective at low humidities. This is because high concentrations are necessary for bactericidal action and these cannot be reached easily at high humidities, when the particles will contain a large amount of moisture (Nash, 1951).

(d) Dry, dust-carried bacteria are much more difficult to kill than organisms from the respiratory tract or from artificially dispersed suspensions of bacteria. Lack of moisture, in which the bactericide can dissolve, may explain this.

#### B. Methods of Dispersal

##### 1 SPRAYING

This is suitable for bactericidal liquids and solutions. The aim is to produce a cloud of minute liquid droplets that are mostly within the aerosol range (0.1 to 50  $\mu$  in diameter). These undergo Brownian movement and, therefore, remain in suspension for a long time. They do not burst on contact with surfaces and, consequently, do not cause wetting. Some transfer the bactericide by direct contact, others evaporate quickly while, from some, the vapour may drift over to the bacteria-carrying particle.

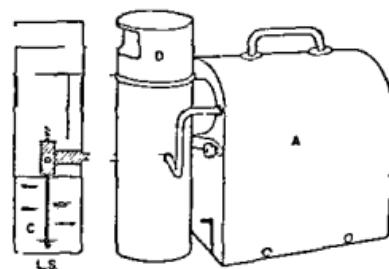


Fig. 24.10 PHANTOMYSER (SEMI-DIAGRAMMATIC)

A—Air compressor B—Jet  
C—Bactericide D—System of baffles  
(Courtesy Aerolot Products Ltd.)

Power driven sprays are desirable. An example is the Phantomyser (Fig. 24.10) in which compressed air is supplied to a fine jet, to atomise the solution, and baffles free the spray from large droplets.

Mists are more likely than vapours to irritate the respiratory tract.

## 2 VAPORISATION

This is the more logical method, if the theory of bactericidal action through the vapour phase is correct. It is applicable to many aerial bactericides. In some cases boiling is suitable e.g. propylene glycol (but see Lidwell and Lovelock, 1948b), for others a cup shaped thermostatically-controlled hot plate has been used, e.g. hexyl resorcinol. It cannot be used for thermolabile substances such as hypochlorites.

### C. Some Problems of Chemical Air Sterilisation

#### 1 DISAPPEARANCE OF THE VAPOUR FROM THE AIR

Aerial bactericides usually disappear quite rapidly. Several factors are responsible, including instability in the atmosphere (e.g. resorcinol, which is oxidised), reaction with substances in the air or on surfaces (e.g. hypochlorous acid is inactivated by phenolic vapours or films of liquid phenolic disinfectants on surfaces), and condensation on, or adsorption by, surfaces (e.g. propylene glycol). Consequently, much more than the theoretical amount of bactericide must be vaporised to maintain the required level in the air (Lidwell and Lovelock, 1948a).

#### 2 UNEVEN DISTRIBUTION

This may give regions of unpleasantly high and ineffectively low concentrations. It can be avoided by a small fan near to the generator.

#### 3 VARIATION IN VENTILATION RATE

Changes in concentration are inevitable when doors are opened. Automatic compensation is possible but very costly. The ideal solution would be a germicide capable of producing an effective concentration at room temperature, because more would vaporise when a sudden increase in ventilation rate reduced the air concentration (Lovelock, 1948).

#### 4 THE PROXIMITY OF EFFECTIVE CONCENTRATIONS TO IRRITANT OR TOXIC CONCENTRATIONS

Bourdillon (1948) states if high killing rates are needed the ratio between the two may be less than 5 : 1.

## EXAMPLES OF AERIAL BACTERICIDES

### 1. Sodium Hypochlorite

This was one of the first substances used. Its thermolability necessitates spraying, and stabilised 0.5 or 1 per cent solutions are suitable. Most aerial bactericides are much more effective in air than in a liquid medium, sodium hypochlorite is exceptional in having about the same activity in both. It is very cheap.

It disappears rapidly from air, is inactivated by phenols and attacks metals, it should not be used if the room contains metal fittings or cabinets, or electrical apparatus. It has a pronounced smell but this quickly fatigues the olfactory sense organs and is not noticeable after a time.

### 2 Phenols

#### (a) Resorcinol

This is easily vaporised and has also been sprayed in glycerin solution. It is effective in very low concentrations, 1 part in 250 million killing approximately 90 per cent of artificially produced infections of vegetative organisms. It is wholly non-irritating but extremely quickly lost from the air.

#### (b) Hexylresorcinol

This has been extensively investigated in recent years (e.g. Mackay, 1952, Darlow, *et al.*, 1958). Like resorcinol, it is non-irritating but is more persistent and is active in greater dilutions (1 part in 1,000 to 5,000 million parts of air). However, disappointing results have followed its use to reduce the incidence of respiratory infections in offices.

### 3 Glycols

#### (a) Propylene Glycol

In this country most interest has been shown in propylene glycol (e.g. Lidwell, Lovelock and Raymond, 1948). For satisfactory bactericidal action the bacteria-carrying particles must contain a very high concentration and, therefore, the atmosphere must be maintained at or near saturation level. To achieve this, large amounts must be vaporised. One part in 2 to 3 million destroys vegetative bacteria and influenza virus under laboratory conditions. When vaporisation is used precautions are necessary to prevent oxidation (Lidwell and Lovelock, 1948b).

#### (b) Triethylene Glycol

This compound is extremely active, a concentration of 1 part in  $2 \times 10^{11}$  parts of air is viricidal and produces virtual sterility of an atmosphere sprayed with

vegetative bacteria in a few seconds. It is non-irritant.

Glycols are less affected by low humidities than other aerial bactericides.

Equipment is obtainable commercially for (i) spraying sodium hypochlorite, (ii) spraying solutions of resorcinol in propylene glycol, and (iii) vapourising hexylresorcinol. The disadvantages of sodium

hypochlorite make it unsuitable for asepsis laboratories but the others are useful in a small room that has no other means of air purification, treatment should begin about  $\frac{1}{2}$  an hour before work starts. The chief disadvantages of aerial bactericides for this purpose are their inactivity against spores and the possibility of contaminating products with the vapours.

### Investigating the Efficiency of Air-sterilisation Methods

#### A AIR SAMPLING

The efficiency of an air sterilisation method is estimated by comparing the number of bacteria or bacteria-carrying particles before, after and, sometimes, during the process. Reliable methods of air sampling are necessary—

##### 1. Settling Plates

Open Petri dishes of nutrient agar are exposed for a suitable time, incubated and the colonies counted. The method depends on the sedimentation of bacteria and is appropriate for an asepsis room because sedimenting organisms are an important hazard. However, it is slow, it fails to collect the very small particles which stay in suspension, and it does not indicate the volume from which the organisms have settled.

##### 2. Impingement Methods

###### (a) ON TO SOLID MEDIA

The particles from a known volume of air are made to impinge on the surface of a solid culture medium. Two of the most important samplers using this principle are—

###### (i) The Bourdillon Slit Sampler

This is widely used in the U.K. Air is rapidly drawn through a very narrow horizontal slit on to the surface of a slowly rotating agar plate only 2 mm below

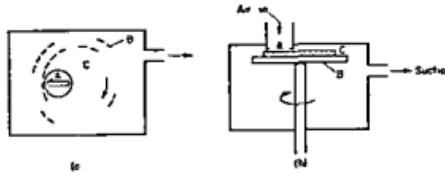


Fig. 24.11 SLIT SAMPLER (DIAGRAMMATIC)

(a) Plan (b) Vertical section  
A—Slit B—Rotating plate carrier  
C—Agar plate

###### of Air-sterilisation Methods

(Fig. 24.11) The high velocity makes the particles impinge on the agar, where they adhere. The collection efficiency is high and under optimal conditions approaches 99 per cent for particles down to about  $1\text{ }\mu$ . The bacterial density of the air can be calculated from the air flow rate (usually  $1\text{ ft}^3/\text{min}$ ) and the exposure time. Variations in bacterial population during the exposure are shown by the distributions of colonies on different sectors of the plate.

###### (ii) The Wells Air Centrifuge

This is an American invention (see Walter and Errera, 1960). A gentle stream of air, at known flow rate, is passed into a rapidly spinning vertical glass tube the interior of which is lined with nutrient agar. Centrifugal force causes the particles to impinge on the agar (Fig. 24.12).

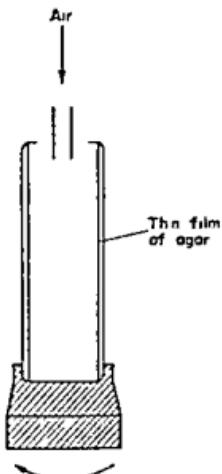


Fig. 24.12 PRINCIPAL FEATURES OF WELLS AIR CENTRIFUGE

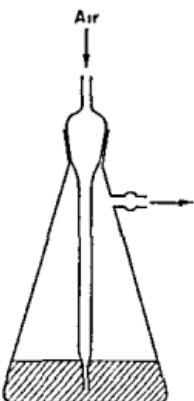


Fig. 24.13 CAPILLARY IMPINGER

## (b) IN TO LIQUID MEDIA

*The Capillary Impinger*

Air, at known flow rate, is drawn by vacuum through a glass capillary into a suitable collecting fluid (Fig. 24.13). The high velocity of the emerging air stream causes particles to impinge on the liquid and become trapped in it. Violent agitation is produced, disrupts the particles and separates the bacteria, consequently, when aliquots of the liquid are plated, the count indicates the number of bacteria present not, as in solid impingement samplers, the number of bacteria-carrying particles.

Performing the count is more laborious than in solid impingement methods, in which the organisms are received directly on the culture medium, but the apparatus is simple in design and use and more suitable when the air is heavily contaminated as, for example, in experimental studies on air sterilisation. If solid media methods are used for the latter, serious overcrowding of the colonies can occur unless inconveniently short exposures are given.

**3 Bubblers**

In these the air is bubbled into a liquid through a glass tube ending in a bulb containing small holes. The air velocity at the points at which the air enters the liquid is low and, therefore, these are not impingement devices. Particles are simply trapped in the liquid. The collection efficiency is relatively low.

**4 Filters**

Henderson (1952) used a microfilter containing a pad of menno wool either mixed with asbestos, or resin-

coated, or treated with acacia. After filtration it was shaken with a liquid. Its collection efficiency for *Bacillus subtilis* was 99.98 to 99.99 per cent.

An ingenious method was devised by Richards (1955). Air was passed through a filter of sodium alginate fibres and, because these are water-soluble, they dissolved when the pad was subsequently added to water. The organisms were liberated more effectively and completely than from insoluble filtering media.

In the United States, the Millipore Filter Corporation have developed a technique for collecting air-borne organisms in which a cellulose-ester membrane filter is used. A pore size of  $0.45 \pm 0.02 \mu$  is chosen, to ensure retention of all the particles. In air sampling it is not sufficient to collect all, or almost all, of the particles—it is equally important to minimise loss of viability during the process. Solid impingement methods are most satisfactory in this respect because the organisms are collected on a nutrient medium. In liquid techniques loss can be minimised by collecting into nutrient fluids. During filtration the air passing over the bacteria in the dry filter causes dessication, and a high loss of viability in vegetative organisms. For this reason, the Millipore Corporation now recommend collection of the organisms in a capillary impinger containing broth, and filtration through the membrane immediately afterwards. Finally, the filter is incubated, in the usual way (see chapter 25), on an absorbent pad saturated with a suitable broth. Afterwards the colonies on its surface are counted.

**5 Electrostatic Samplers**

These make use of electrostatic precipitation for collection. Usually a layer of agar is used as the collecting electrode. They are more complex but no more efficient than most of the methods mentioned above.

(For further information see Salle (1961)—general account, Bourdillon, Lidwell and Thomas (1948), Schuster (1948) and Mackay (1952)—modifications to the slit sampler, Andersen (1958) and Lidwell (1959)—separate collection of different sizes of particle, Kluyver and Visser (1950) and Henderson (1952)—capillary impingement, Houwink and Rolvink (1957)—electrostatic precipitation)

**B THE EVALUATION OF FILTERS****1 Bacteriological Testing**

This type of test has the advantage of showing directly that a filter can remove micro-organisms efficiently.

Essentially it involves mixing bacteria with the upstream air and sampling before and after passage through the filter. The following are important features—

(a) It must be possible to vary the air velocity because of its marked effect on particle retention mechanisms.

(b) The filter must be mounted so that by passing at the edges is impossible.

(c) The bacterial infection can be an aerosol containing vegetative bacteria (resembling respiratory infection), or a spore-infected dust (simulating dust-borne contamination). The aerosol will evaporate quickly leaving naked or near naked bacteria that, because of their small size, will test the filter severely. A suitable vegetative organism is *Serratia marcescens* (see "Tests on Filters for Liquids") and a convenient source of spores is *Bacillus subtilis* (Sykes and Carter, 1953).

(d) Sampling is usually done with slit samplers. Upstream contamination is adjusted to give a countable number of colonies on the prefiltration plates, and downstream sampling is timed to collect approximately the same number of organisms per plate as on the upstream plates.

Methods of this kind have been used for the comparison of filtering media for asepsis rooms (Sykes and Carter) and to investigate the suitability of mineral wools for providing fermenter air (Terjesen and Cherry, 1947; Cherry, McCann and Parker, 1951). Heat and electrostatic precipitation can be studied in a similar way by substituting the appropriate equipment for the filter.

## 2. The Methylene Blue Test

This test, which is described in detail in BS 2831 1957 is used primarily for high efficiency and absolute filters. A methylene blue solution is atomised and the spray discharged into an airstream passing through a duct containing the filter. The upstream part of the duct is long enough to ensure complete evaporation of the droplets before the spray reaches the filter. Therefore, the air arriving at the filter contains fine suspended particles of methylene blue. The pre- and post filter air is sampled onto white filter paper which is then steamed to bring out the colour. The optical densities of the stains are compared with a densitometer. Downstream samples are taken over longer periods of time to obtain a paper of approximately equivalent intensity to one of the upstream samples. Then if, for example, the papers from a  $5 \text{ cm}^3$  upstream and a  $5,000 \text{ cm}^3$  downstream sample match exactly, the staining power of the

filtered air is only  $1/1,000$  of the unfiltered air and, therefore, the methylene blue penetration is 0.1 per cent. The relation of this test to the suitability of a filter for removing bacteria is discussed in connexion with types of filter (pp 412-415).

## 3 The Sodium Flame Test

Sodium chloride is sprayed and atomised, a cloud of particles of about  $0.3 \mu$  in diameter results. The sodium content of the unfiltered and filtered air is estimated by the intensity of its yellow lines in the hydrogen flame. This method is quicker than the methylene blue technique.

## C. EVALUATION OF AERIAL BACTERICIDES

Methods of air sterilisation in which the process is over almost immediately (e.g. filtration, electrostatic precipitation and heat) can be evaluated fairly simply by finding, as in the bacteriological method for filters described above, the percentage of living organisms remaining after treatment.

Ultra-violet radiation and chemicals are used continuously and act more slowly. Therefore, it is more realistic to determine the death rate of the organisms from counts made at intervals. A test chamber, at controlled temperature and humidity, is sprayed with the organism and the air immediately sampled for counting. Then the bactericidal agent is dispersed and sampling is continued at definite time intervals. The death rate is calculated from the results.

The problems of testing aerial disinfectants were fully reviewed in a report by the Aerosols Panel of the British Disinfectant Manufacturers Association (Aerosols Panel, 1949) and commented upon by Baker (1949). Their recommendations became the basis for a British Standard Specification (BS 2796 1956).

## D PLANT TESTS FOR FERMENTER AIR

An antibiotic fermentation lasts several days and there is ample time for a few contaminants to multiply into a population that can seriously affect the yield. Therefore, the presence of even an occasional organism in the air is dangerous. Since thousands of cubic feet of air pass through the medium every hour, the sample volume necessary to show that these infrequent organisms are absent is much greater than normal samplers can handle.

One method of dealing with this problem is to use the bacteriological test method for filters with a

very heavy contamination of the unfiltered air. Then, if the filter performs satisfactorily, it is assumed that it will remove the lighter bacterial content of a much larger volume of normal air.

The alternative is a plant test (Cherry, McCann and Parker, 1951) in which a fermenter of medium at pH and temperature favourable to the growth of

contaminants is aerated for several days longer than in a normal fermentation and samples are examined for sterility at intervals.

(For a review of all aspects of air sterilisation see Sykes (1964), and for further information on the sterilisation of air for fermenters see Steel, 1958, Sykes and Carter, 1954, Whitmarsh, 1958)

### Other Aspects of Asepsis Room Ventilation

#### 1 CONTROL OF CONTAMINATED AIR

A good ventilation system does not merely admit a steady flow of clean air, it also prevents the accumulation of contaminants stirred up or respired into the atmosphere and stops the entry of dirty air through open doors.

##### *Accumulation of Contaminants*

This is prevented by admitting clean air at a rate sufficient to replace the atmosphere frequently. Usually about 10 air changes per hour are considered adequate.

There are two main types of ventilation system. In the first, the displacement system, air is admitted through carefully placed diffusers, on the ceiling, these spread the air out over the top of the room whence it gradually moves down, forcing the old air out of ports in the bottoms of the walls, i.e. in a way similar to the non-turbulent displacement of air by steam in a large autoclave. It is most efficient if the clean air is warmer than the room air, but it is easily upset by activity in the room and by upward air flow created by Bunsens, radiators etc. The alternative is the turbulent system in which the air is admitted turbulently through suitably shaped louvres. Rapid mixing of purified and stale air takes place and, therefore, localised areas of high contamination (e.g. around workers) are quickly dispersed. The method is not upset by activity and heating apparatus. In the displacement method, the positioning of the air inlets and outlets is critical, this is not so with the turbulent system. However, with the latter there is more danger of sweeping contaminants into open screens because of the greater air movement. There is insufficient evidence to show which of these methods is most suitable for an asepsis room (but see the work of Blowers and Crew (1960) on the use of the two systems for operating theatres).

##### *Entry of Unsterile Air*

Dirty air is prevented from entering door cracks and openings by pressurisation. This can be achieved—

(a) When recirculation is used—by removing less air than is put in

(b) By using fresh air only and allowing all the air to escape through weighted flap valves. The extent to which these open depends on the internal pressure. When a door is opened and the room pressure drops the valves automatically close to restore the correct level of pressurisation, and prevent the entry of dirty air, at the door opening. This is the better method because, with recirculation, there is a marked inflow of air when a door is opened, particularly near to the floor, unless the room has exceptionally high pressurisation. Pressurisation may be checked by generating smoke and observing the direction of airflow.

#### 2 MAINTENANCE OF THE TRUNKING

The trunking of the ventilation system must be accessible and contain doors through which it can be cleaned regularly.

#### 3 AIR SUPPLY TO SCREENS

The supply of bacteriologically clean air to screens has the advantages of—

- (a) avoiding the expense of providing high quality air to the whole room,
- (b) forcing unsterile air away from the working space and reducing the chance of contamination by staff
- (c) allowing large numbers of air changes (e.g. 1/min), because only small volumes are needed

Rugby (1961) has reported the successful use of this method, air filtered through slag wool is admitted to the screens through ports in stainless steel benches, metal mesh diffusers are fitted over the ports to prevent excessive turbulence.

### SIMPLE EXERCISES IN ASEPTIC TECHNIQUE

#### Reducing Contamination

The elaborate measures described in the previous sections are intended to supplement, not to replace

the skill of the operator. In fact, with practice, short exercises involving only simple procedures can be performed successfully with relatively few precautions.

It is useful to learn and practise aseptic technique under less than ideal conditions. Then, the poor results that follow carelessness, slowness and lack of dexterity act as spurs to improvement. When proficiency has been acquired, considerable satisfaction can be obtained from the knowledge that, in practice, similar work would be carried out under superior conditions.

During this training period the following are adequate methods of reducing contamination from the sources listed at the beginning of this chapter:

### 1 Atmosphere

Protect the working area with a screen. Reduce air movements to a minimum by—

- (a) Before starting work, collecting near to hand everything needed for the exercise
- (b) Remaining seated throughout the exercise

### 2 Breath

Raise the chair seat so that the face is well above the screen opening. Shack type screens allow the head to be bent well forward over the sloping front, to reduce further the risk of breath reaching the contents.

### 3 Hair

The methods for excluding breath minimise contamination from the hair. However, if it is frequently necessary to brush or shake hair from the face a suitable cap should be worn.

### 4 Clothes

Preferably, wear one of the styles of gown recommended earlier. Any alternative must be long and kept fully buttoned up. It need not be sterile but should be freshly laundered, if unsterile, the sleeves must be rolled up to or, preferably, cut short at, a point well above the elbows.

### 5 Working Surface

Swab the bench top with disinfectant. A large (12 in square) tile, that has been flamed, or sterilised in an oven or autoclave, can be used as a dry surface for sterile equipment.

### 6 Hands

Wash and disinfect.

### Preparation for Working

Tasks involving walking about or vigorous movements must be done first and as much as possible of the handling of unsterile articles completed before the hands are washed.

There are many equally satisfactory ways of performing exercises in aseptic technique and there is a variety of equally suitable types of equipment. It is impracticable to mention all of these and, instead, methods that in the author's experience have been particularly successful will be used to illustrate principles and stimulate a critical approach to the subject.

It will be assumed that the working surface is against a wall on which the service points are mounted. Beneath the bench, to the right, is a waste bucket that can be swivelled out. In front is a movable trolley unit and an adjustable chair. On the bench top is a screen (Fig. 24.14).

In a drawer of the unit is a metal box (which can be dry-heat sterilised occasionally) containing small equipment (e.g. 2 pairs of heavy, blunt nosed forceps, an ampoule file, several sizes of teat, a wooden-handled spatula with a stainless-steel blade, a glass-marking pencil and several gate clips).

In the cupboard of the unit are—

The large tile

A Bunsen burner with a pilot jet

A lidded swab-jar containing large absorbent cotton-wool balls

A dust proof bottle (4 oz is suitable), with a permanent ceramic label, containing 1 per cent of cetrimide and another containing 75 per cent alcohol

Two stainless steel gallipots (tiny bowls) or 100-ml wide-mouthed beakers

Stainless steel pipette and test tube racks

As far as possible, equipment should be chosen for its freedom from dust-collecting nooks and

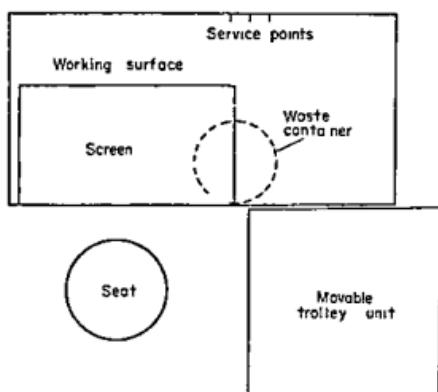


Fig. 24.14

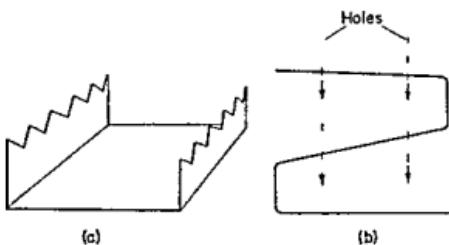


Fig. 24.15 WELL DESIGNED ASEPTIC EQUIPMENT

(a) Pette rack (b) Test tube rack (end view)  
Both are made simply by bending stainless-steel sheet

crannies Two examples of good, yet simple, design are shown in Fig. 24.15

#### Procedure

- 1 Put on the gown
- 2 Study the exercise, decide the equipment and materials needed and write any labels. If possible do this outside the laboratory, then the gown can be put on afterwards.
- 3 Position and brake the trolley unit in its working position. Remove its contents to the top
- 4 Adjust the chair height, swivel it gently to minimise air disturbance
- 5 Arrange the chair in the working position and take care not to move it thereafter
- 6 Swing out the waste bucket
- 7 Collect all the articles required for the exercise. Use a basket so that only one journey is required. Put the basket on the trolley
- 8 Attach and light the burner, preferably with a battery lighter. Never use paper ignited from another burner, it scatters particles into the air and vigorous blowing or shaking is necessary to extinguish it. Place the burner as near to the screen as possible without danger of damaging the plastic, and right at the edge of the bench. No advantage is gained from having it inside the screen where it will cause considerable air movement and may melt the plastic. In the recommended position it is only a very short distance from burner to screen and there is very little likelihood of contamination taking place as this is traversed
- 9 Sit down
- 10 Place the swab jar, one gallipot and the bottle of cetrimide conveniently to hand at the right of the Bunsen Swab bench surface under screen
- Cetrimide is one of several antibacterial agents suitable for this purpose. It is active against a wide range of bacteria, its effect persists for several hours
- it is detergent at the concentration used and its solutions are colourless, odourless, non irritant and non-toxic. Its activity is reduced or destroyed by soaps and other anionic surface active compounds because these combine with its active cation. Therefore, laboratory staff should use non ionic detergents for cleaning the benches in case a film is left on the surface. Cetrimide makes the skin of some people excessively dry, they should use another solution such as chlorhexidine 0.5 per cent, or Solution of Chloroxylenol 25 per cent.
- Swab methodically from side to side of the screen, working from back to front and taking care not to sweep from an untreated to a treated area. If necessary use a second swab, do not remoisten the dirty one or leave it lying about—drop it into the waste bucket
- In practice, swabbing the inner surfaces of the screen itself is of little advantage. It makes the air excessively damp (see 'Weighing') and, with some solutions affects visibility, looks messy and gradually dulls the plastic. A better procedure is to make screen washing a part of the evening laboratory cleaning routine. If swabbing is preferred, alcoholic solutions should be used
- 11 Swab the test tube rack and put it to the right, inside the screen
- 12 Fix identifying labels on the tubes (see Exercise 24.1) or mark them with a glass writing pencil. Labels are preferable because pencil marking is either easily rubbed off by, and on to, the fingers or is only removable with great difficulty, to the annoyance of cleaning staff. Chinagraph pencils are an example of the removable type but are widely used. The blue colour is superior to any other and gives excellent results if the point is sharp, the slightest trace of grease causes difficulty and must first be removed with a dry swab. Warming the tube helps, but the tendency is to heat too much, the pencil point melting into an illegible blob.
- Labels that need moistening must not be licked. Small self adhesive labels are superior. They should be purchased attached to backing sheets (i.e. not in rolls) from which removal is unnecessary until they have been written, this is a great convenience because trying to hold still and, at the same time, write on a tiny label can be very frustrating. When used on tubes requiring incubation there is a tendency for separation to occur at the edges but they rarely come off altogether. Their main fault is the film of adhesive left on the tubes after removal which is rather difficult to clean off.
- 13 If the tile has been sterilised in an oven or autoclave it will be wrapped and need not be opened

until later If it is to be flamed a suitable procedure is—

Place it near to the burner with a portion overlapping at the edge of the bench.

Invert the burner and pass the flame slowly from side to side over the whole surface Repeat more quickly to destroy any organisms that have fallen on the parts that were flamed first. Keep the gas tubing off the flamed surface

With fingers under the overlapping part, pull the tile from the bench until both hands can be placed below Then lift it into the screen and arrange it near to the back, at the left, but not quite touching the walls

The preparation of the tile is done at this stage because it involves handling the relatively dirty burner and tubing If air movements are few very little contamination of its surface will take place before it is used. The pipette rack is prepared at this stage unless it is small enough to be handled with forceps, when it can be left until later It should be flamed, not swabbed to prevent contamination of pipettes with antiseptic

14 Swab the top of the lid of the swab jar because before it is used again the hands will have been prepared.

15 Arrange the rest of the screen equipment near to the Bunsen This includes the alcohol and the second gallipot, and the tray of small equipment The items in the latter should overlap its edges so that they can be picked up easily without fumbling inside the tray

16 Arrange the articles from the basket conveniently on the unit The final suggested arrangement is shown in Fig 24 16

Careful study of the order in the above scheme will confirm that—

(a) Operations involving much movement have been completed early, e.g.—

Removal of the equipment from the unit.

Adjustment of the chair

(b) Movements have been reduced to a minimum, e.g.—

All articles for the subsequent exercise are collected in a basket to avoid several journeys The operator sits as soon as possible

(c) Equipment that is relatively difficult to keep clean and therefore, is most likely to contaminate the hands, is arranged or used before scrubbing up, e.g. the chair, waste bucket and burner

17 Preparation of hands and arms (Scrubbing-up)

#### (a) ATTENTION TO NAILS

The succeeding procedures cannot be carried out effectively if the nails are long They must be trimmed carefully and scrubbed free from visible dirt

#### (b) WASHING

Thorough washing with soap and water for 3 minutes removes most of the transient bacteria but even after vigorous scrubbing for 10 minutes about 30 per cent of the resident bacteria remain

The resident flora is difficult to remove because most of the organisms are deeply embedded in the pores, but since forcible treatment is necessary to bring them to the surface they are unlikely to cause contamination during asepsis Therefore, there seems little advantage in prolonging pre-asepsis washing beyond the point at which the transient and surface-resident bacteria have been removed. In addition, the use of a brush, except for the nails, is undesirable because it causes unnecessary liberation of the deep residents

Nevertheless, although it is hard to justify an extended scrubbing time to remove organisms that are unlikely to be a hazard, the further reduction of this part of the flora by a quicker method—the use of a disinfectant—is a useful precaution

#### (c) DISINFECTION

Very few substances meet the requirements for an ideal skin disinfectant, namely—

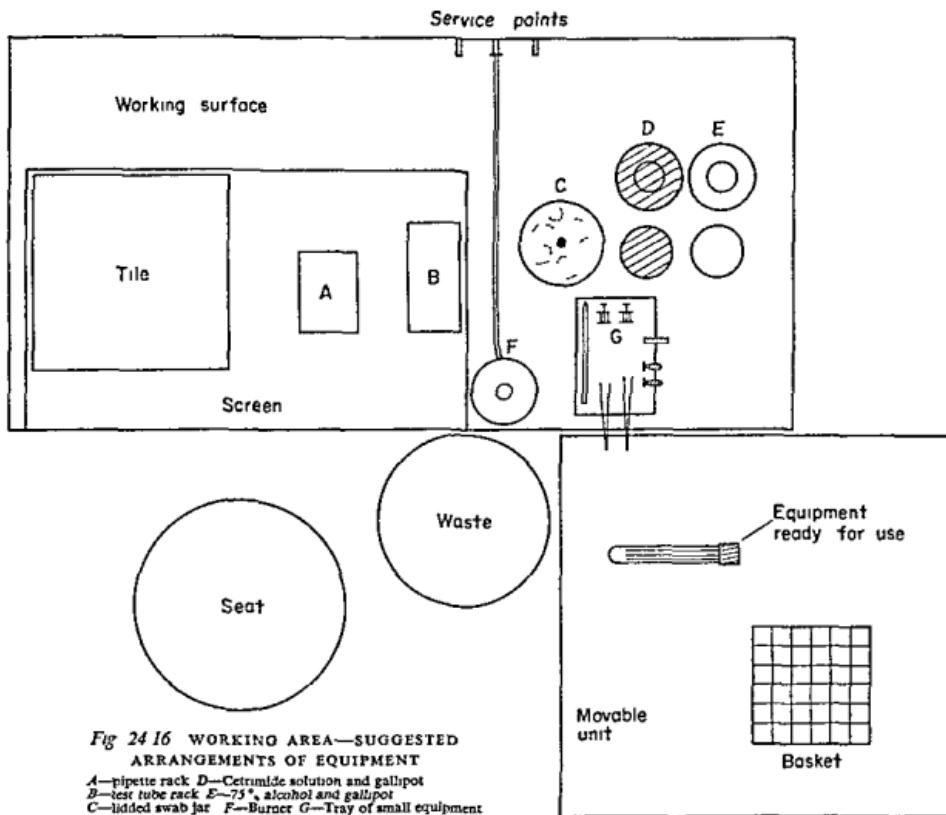
- (i) High germicidal activity against the transient and resident flora.
- (ii) Rapidity of action.
- (iii) Persistence of activity
- (iv) Compatibility with soap
- (v) Freedom from toxicity, irritancy and the tendency to produce sensitisation
- (vi) Non staining

Two compounds that give good results (see, for example, Lowbury, 1961) are hexachlorophane and ethyl alcohol, they are used in different ways—

#### *Hexachlorophane*

In this case, the technique is to wash regularly (3 or 4 times a day) with a soap or cream containing the substance

The activity of hexachlorophane is largely confined to Gram positive bacteria and is slow but, unlike most disinfectants, it is effective when incorporated in soap, from which it is deposited on the skin as a persistent, protective film. It is non-irritant, non toxic, non staining and does not cause sensitisation.



**Fig. 2416 WORKING AREA—SUGGESTED ARRANGEMENTS OF EQUIPMENT**

A—pipette rack D—Cetrimide solution and gallipot  
 B—test tube rack E—75% alcohol and gallipot  
 C—lidless swab jar F—Burner G—Tray of small equipment

The most important formulations are soap tablets containing 2 per cent and an emulsion containing 3 per cent plus a detergent (pHisoHex, Bayer Products Ltd). The emulsion is particularly effective and is suitable for persons sensitive to soap. Jones (1952) found that regular use of the soap caused a marked reduction of the skin flora of staff employed in asepsis filling rooms. His results have been supported by Lawrie (1952), Lord, Thomas and Parker (1952) and Gump and Cade (1953).

Regular use of a hexachlorophane preparation for about 4 days reduces the skin flora to the level obtainable by a 10-minute scrub.

#### Ethyl Alcohol

In this method, the washed hands and arms are

dried and then treated with 70 per cent alcohol for 2 minutes, preferably by immersion.

Alcohol kills vegetative bacteria quickly. It has no effect on spores but this is not a serious deficiency because there should be no spores on the skin after efficient washing. It is used within the range 70 to 75 per cent because lower and higher concentrations are very much less effective. It evaporates quickly and, therefore, its action is only transient. The addition of 0.5 per cent chlorhexidine slightly improves the antibacterial activity and gives some persistence, but this substance, like cetrimide, is inactivated by soaps and, therefore, the rinsing after washing must be particularly efficient.

The preparations used as skin disinfectants do not kill pathogenic spores, e.g. those of *Clostridium spp.* Washing is the means by which these potentially

dangerous members of the transient flora are removed and, therefore, it cannot be replaced entirely by disinfection. It also assists the latter by freeing the skin from dirt, grease and fat which would interfere with the efficiency of the antibacterial agent.

To summarise—

*Hexachlorophane Method* Use a hexachlorophane preparation regularly and just before asepsis. For the latter, first attend to the nails, then work up a good lather, wash this off and then repeat, this time working the lather into every part of the hands and forearms and giving special attention to the nails and knuckle folds. Parts of the right hand are often neglected by right handed people. After 3 minutes,

rinse well, holding the hands in the air, because in this position micro-organisms will be rinsed away from them. Do not turn off the taps with the hands. Dry the skin thoroughly, either on sterile towels or in hot air.

*Alcohol Method* Attend to the nails and wash the hands and arms in soap and water for 3 minutes. Dry, this is essential to avoid diluting the alcohol with residual moisture. Rub 70 per cent alcohol into the skin for 2 minutes, keeping the surface wet with it all the time.

(For further information on skin disinfection see Extra Pharmacopoeia (1955), Annotation (1958), Pritchard (1952), Verdon (1961), Walter (1954) and Williams *et al* (1960))

## BASIC EXERCISES

The aim of aseptic technique—to process materials without contaminating them—is not difficult to fulfil because the basic operations are very easy and even the beginner can soon perform a variety of exercises with a high degree of success if three rules are constantly followed—

- 1 Use a non touch technique whenever possible, for example, handle small articles with flamed forceps and when sterile apparatus must be touched, do this as far as possible from the part that will later come into contact with a sterile liquid or powder.
- 2 Reduce air disturbance to a minimum, for example, by keeping the body as still as possible and avoiding sharp movements of the hands and arms.
- 3 Refuse to allow interruption.

Techniques are practised using a liquid medium in which bacteria will grow readily, e.g. peptone water or nutrient broth. Afterwards the containers are incubated and contamination is detected by the turbidity produced. Unsatisfactory results are less serious if they can be explained. Therefore, all containers should be numbered and a mental note (later to be transferred to paper) made during the exercise of any in which a fault in manipulation might have caused contamination. Then, when they are examined after incubation it will often be possible to give the reason for a bad result. This practice also helps to show the relative seriousness of different mistakes in technique, some of which will almost certainly not cause infection.

### A Aseptic Transferences

The object of the four exercises in this group is to transfer a liquid aseptically from one container to

another. Each illustrates an important technique used regularly in testing injections for sterility.

#### EXERCISE 241

#### *Transference of a Liquid from One Tube to Another using a Pasteur Pipette*

##### *Applications to Sterility Testing*

- Inoculations of controls with organisms,
- Subculture of unreadable or doubtful tubes

It may seem contradictory to give as examples of aseptic technique two procedures that either involve or, in the second case, may involve the transfer of bacteria. However, it is equally important not to contaminate the containers with unwanted organisms in these cases as it is when sterile liquids are being transferred.

In fact, it is impossible to justify the presence in an asepsis room of containers known or suspected to contain large numbers of micro-organisms since an accident or faulty technique would negate the elaborate precautions taken to ensure aseptic conditions. Therefore, operations involving the handling of live organisms are performed in a separate room where, because they are regarded as bacteriological rather than aseptic techniques, a screen is rarely used. Consequently, it is essential to be able to carry out this type of transference skilfully.

#### STERILE MATERIALS REQUIRED

12 tubes of broth Label these *A1* to 6 and *B1* to 6. Usually six replicates are sufficient to show that an exercise has been mastered.

2 tubes of Pasteur (capillary) pipettes These pipettes are made from glass tubing (often 5 mm external diameter and 20 cm long) by drawing the centre into a fine capillary and when cool, filing and breaking it in the middle (Fig. 24 17). The wide ends of the two resulting pipettes are plugged with non absorbent cotton wool and the excess is cut or burned off because if fibres become trapped between the mouth of the teat and the glass an air leak results which prevents proper use of the teat. Alternatively, the wool may be pushed right into the end, this makes removal difficult and, usually, necessitates discarding the pipette after use.

#### PROCEDURE

1 Sit down without touching the chair with the hands

2 Flame the tips of a pair of forceps Flaming is often used in asepsis for sterilising such items as the tips of forceps, blades of spatulas, necks of tubes and bottles and the ends of glass rods To ensure sterility the part must be made very hot by holding it in the flame for about 20 seconds. Rest the forceps on the edge of the tile or on the pipette rack, with the tips in the air

3 Place a tube of pipettes quite horizontally in the left hand (L H) keeping the latter well under the screen and 6 in or more from the bench This horizontal position, in which it is almost impossible for contamination to drop into the mouth of the tube, is obtained by gripping the tube tightly with the length of the thumb and first finger and using the middle finger for support, it is an advantage to push the tube firmly against the inner edge of the palm (Fig. 24 18)

4 Remove the cap with the little finger and palm of the right hand (R H) and, taking care not to touch the cap on the bench, pick up the forceps and use them to grasp the end of one of the uppermost pipettes and draw it about 2 in from the tube Lay down forceps and, holding extreme end of pipette, withdraw it with the fingers and rest it on the rack.

5 Recap the tube Its neck need not be flamed Contamination is very unlikely if the tube has been held as suggested, the cap has been

(continued overleaf)



Fig. 24 17

*Exercise 24 I continued*

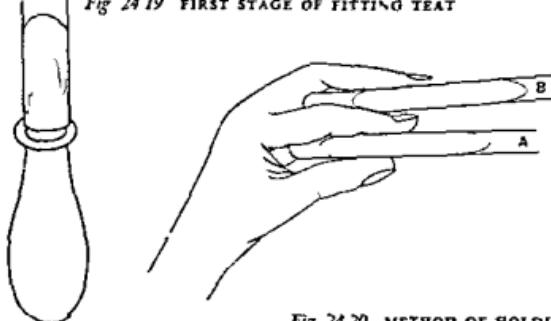
kept facing the bench, and movements have been unflurried. Transfer the tube to the R.H. and place it in the rack. Notice that the L.H. is not used to do this because it would have to move right across the screen causing more movement than the method recommended.



*Fig. 24.18 HORIZONTAL METHOD OF HOLDING TUBE OF CAPILLARY PIPETTES (PIPETTES OMITTED)*

6 With the thumb and first finger of the L.H. pick up the pipette holding it as near the plug as possible and above the hand. Fitting a tight teat often jerks the pipette, and if it is below the hand the tip may be knocked against the palm. Attaching the teat is a little difficult if it fits well. One useful method is to slip its lower edge under the end of the pipette (Fig. 24.19) and hold this tightly in place with the middle finger (L.H.) while stretching the upper edge of the lip over the glass with the thumb and first finger of the R.H. The fingers of the L.H. must not be allowed to move appreciably towards the pipette tip. Replace the pipette on the rack.

*Fig. 24.19 FIRST STAGE OF FITTING TEAT*



*Fig. 24.20 METHOD OF HOLDING TUBES  
View seen as the eye looks directly down*

7 The transferences will be made from the *A* to the *B* tubes. Place *A* in the L.H. in approximately the same position as was used for the pipette tube but slightly less horizontal to avoid spilling the broth. Put *B* between the 1st and 2nd fingers, also almost horizontal. With the tubes in these positions the operator should be looking at the back of his L.H. (Fig. 24.20). This is a good method of holding tubes for aseptic work because not only does it reduce the chance of contamination but it also makes the complete contents of the tubes visible, an advantage when anaerobic media, which have to be inoculated at the bottom, are being used. Any difficulty in adopting the suggested grip usually disappears.

when it is realised that hard glass bacteriological tubes will not break when held tightly

8 Pick up the pipette with the thumb and forefinger of the R H and with the same hand remove the caps of A (as above) and B (between the little and 4th fingers). Expel the air from the teat. If expulsion is left until the pipette is in the broth, air will be bubbled through the liquid, and although this air is unlikely to be contaminated, since any from the teat will be filtered by the plug, the practice is undesirable because it could be serious with anaerobic media, where a low oxidation reduction potential must be maintained.

9 Put the tip in the liquid and draw up to the mark. In sterility testing it is not essential to use an exact volume provided it exceeds a specified minimum but a considerable excess must not be added because when the solution contains an inhibitory substance the test medium may not contain sufficient neutralising agent to overcome the unusually large amount transferred. Therefore, the volume should be fairly accurate, and this can be achieved by marking the pipette with a file at a suitable volume, which is usually 1 ml. This calibration takes very little time if the capillaries are cut to the same length and an ampoule cutting device with a gauge (Fig. 23.9) is used to make the mark.

10 Remove the pipette from the liquid, suck the contents away from the tip but not up to the plug, and touch off the residual drop on the inside of the tube. The last two operations prevent broth dropping from the pipette as it is moved to the other tube. It is extremely important to avoid this when suspensions of bacteria are being handled.

11 Transfer the pipette to tube B, gently expel the contents, remove the residual drop and withdraw the pipette. Replace the caps before the pipette is put down. Remove the teat and leave it under the screen for the next transference.

12 Repeat the exercise a further 5 times using a fresh pipette for each.

If capped tubes are used and handled as above it is unnecessary to flame their necks. However, if this precaution is considered advisable it is best left to the end of the exercise when each tube can be taken, in turn, to the burner, uncapped and flamed in the usual way. If the open tubes are brought out of the screen immediately after the completion of each transfer there is a risk that on the way organisms will be scooped, or will fall, inside. Also this operation is difficult to perform smoothly because it involves passing the L H right across the body which must be moved back to make room. Plugged tubes must be flamed before and after use unless the plugs have been protected by individual paper covers—a practice common for flasks but not for tubes. During storage, organisms may be deposited on the glass very close to the tube mouth and, during manipulations, others may be liberated from the plug. The flaming is most easily performed if another burner is available at the left of the screen, then there is no need to carry the tubes across the body and no danger from scooping because the closed ends of the tubes are foremost when they are moved out to the flame.

13 Transfer the tubes to an incubation basket of suitable size. If it is not subdivided, it should contain wool in the bottom to prevent the tubes from slipping over. Take care not to dislodge the caps during handling and never try to lift a container by its closure. Incubate at 31°C for 48 hr. Examine for turbidity and try to explain any unsatisfactory results.

**EXERCISE 24 2**

*Transfer 1 ml from an Ampoule to a Tube using a Pasteur Pipette*

*Application to Sterility Testing*

Testing of single-dose containers of small volume

**STERILE MATERIALS REQUIRED**

6 tubes of broth, label these 1 to 6

Six 2 ml ampoules, unlabelled because marks and labels detach in the solution used to sterilise the outsides. Keep them in a definite arrangement under the screen and, just before incubation, number them to correspond with the appropriate tubes

A tube of Pasteur pipettes

A capped specimen tube containing plugged Durham tubes

**NEW TECHNIQUE INVOLVED Opening of Ampoules**

There must be a very low probability of accidental contamination in sterility testing because infection could lead to the rejection of a batch of sterile containers. One stage at which contamination can take place and at which particular care is necessary is the opening of the container. With ampoules this involves the following techniques

*Sterilising the Outside*

Several methods have been suggested—

(a) *Flaming*. This must be done cautiously because the glass must not become so hot that organisms inside are destroyed. Although it is unlikely that an internally contaminated container would be made sterile by over flaming, the method is not entirely satisfactory because of the possibility that the microbial content before and after may be different. This is not a serious objection but other methods are superior in this respect.

(b) *Ethylene Oxide Gas (q.v.)* Except that rather elaborate equipment is required, this is an excellent solution to the problem.

(c) *Washing, followed by Immersion in 75 per cent Alcohol*. Contaminants are detached from the surface by a thorough washing in warm detergent solution, the particular aim is to remove spores (see 'Scrubbing up'). Then, after several rinses, the last in sterile water, the ampoules are dipped in 75 per cent alcohol and finally transferred to another container of the same solution until required for testing. The dipping avoids dilution of the final alcohol with rinse water on the ampoule surface.

*Opening*

The normal method of filing at the constriction and snapping off the top is not entirely satisfactory. Usually there is a slight vacuum inside, because the air in the neck was hot at the time of sealing, and, therefore, if the neck is broken sharply, air rushes in and may carry contamination with it. Also it is difficult to make a clean break without holding the ampoule in the fingers. As these will have touched unsterile articles it is undesirable to bring them as close to the point of opening as would be necessary, for example, with small ampoules. Also, since the complete neck is removed, the opening of the body cannot be protected with a Durham tube, a metal cap is suitable for large ampoules but even the smallest cap swallows up a small one and makes it difficult to pick up,

unless the cap is shortened. Nevertheless, these disadvantages are largely outweighed by the unreliability of alternative methods which often cause impatience and anxiety and, in consequence, many extra movements. Some of the alternatives are—

- (a) Round  $\frac{1}{4}$  of the circumference of the upper part of the neck a deep file mark is made. This is touched with either a thick wire, curved to fit the ampoule and made red hot, or a hot glass rod.
- (b) A file mark is made, as above, the neck is heated and then a drop of 75 per cent alcohol is put on the mark.

When successful, these methods produce a clean crack through which air enters slowly and is unlikely to carry contaminants. The top of the neck can be gently removed with forceps and, usually, the remainder is long and thin enough to be covered with a Durham tube.

#### PROCEDURE

##### (i) Using First Method of Opening

1 Before scrubbing up, sterilise the outsides of the ampoules by the alcohol method.

2 Flame a metal ampoule file and put it on the tile so that it overlaps slightly and can be picked up easily later.

3 Remove the ampoules from the alcohol with flamed and cooled forceps, file the neck of each.

4 Flame and invert on the tile six  $\frac{1}{2}$  in size metal caps that have been cut down to about half their original length. Take care, because aluminium caps melt if flamed excessively. Alternatively, they may be dry heat sterilised previously, in a capped specimen tube or screw-capped jar.

5 Pick up the ampoules one by one, hold them almost horizontally with the fingers and thumbs, as far as possible from the constriction, then break off the neck. Immediately, pick up a metal cap and place it over the ampoule, returning the latter to upright only when it has been safely covered.

From this point the procedure is essentially the same as for Exercise 24.1. It will be found easier to hold the ampoule, rather than the tube, between the thumb and first finger of the L.H. during the transfer.

##### (ii) Using Alternative Methods of Opening

1 to 3 These are the same as stages 1 to 3 of method (i), but make a file mark about  $\frac{1}{4}$  of the way round the upper part of the neck of each ampoule.

4 With flamed forceps remove six Durham tubes from their tube and place them on the tile. (Note that the tile is being used only for articles that are certainly sterile.) Cap the specimen tube and place it at the back of the screen. (Always keep the screen tidy but do not make unnecessary movement producing excursions outside.) Pick up a Durham tube with forceps, the plug is the most convenient part to grip as the tubes are too light to separate from it. The fingers cannot be used for such a small article without considerable risk of touching the tile. Hold the bottom of the tube with the L.H., remove the plug with forceps and immediately invert the tube on the tile. Repeat with the rest of the tubes but keep them well apart on the tile so that when one is picked up the others are not knocked over.

*(continued overleaf)*

**Exercise 24.2 continued**

5 Crack the first ampoule, then hold it almost horizontal and break off the top with forceps. Immediately cover the neck with a Durham tube. Repeat with the other 5. Then continue as in Exercise 24.1 above.

Notice that as far as possible all the ampoules are subjected to one operation (e.g. filing, opening, transference) before the next is started. This reduces movement and avoids frequent returns to less clean stages.

**EXERCISE 24.3**

*Transfer 1 ml of Sterile Broth from a Mark I Clinbritic Bottle to a Tube using a Pasteur Pipette*

*Application to Sterility Testing*

Testing of multi-dose containers

**STERILE MATERIALS REQUIRED**

6 tubes of broth labelled 1 to 6

6 small Clinbritic bottles containing 10 ml of broth labelled 1 to 6.

A tube of Pasteur pipettes

**NEW TECHNIQUE INVOLVED** The Handling of Multidose Bottles

**PROCEDURE**

1 As the bottles are put under the screen loosen their bakelite caps until they can be lifted as easily as tube caps.

2 Lift off the bakelite cap of the first bottle, swab the rim of the cap with 75 per cent alcohol and stand it, rim down, on the back of the tile. It is put at the back because it cannot be considered sterile, but if it were placed on the bench it might pick up cetrimide solution which, in turn, might be deposited on the rim of the bottle through careless technique at the next stage. From there it could contaminate the pipette or broth and lead to inhibition in the tubes.

3 Swab the rubber cap, plastic ring and neck of the bottle with 75 per cent alcohol. With forceps split off the ring and flick up the flange of the skirted cap. Pull out the cap, it is easier to do this with forceps if the bottle is held firmly on the bench, but immediately afterwards the bottle should be tilted to protect its mouth, and the bakelite cap replaced at once, taking care to avoid contact between its rim and the bottle neck. Repeat with the other bottles, they are then ready for the transfers.

Removal of the sealing ring of the Mark II bottle and the antibiotic vial is difficult, and these containers are best sampled with a syringe using the method described in Exercise 24.4.

**EXERCISE 24.4**

*Transfer 10 ml of Sterile Broth from a Transfusion Bottle to a Bottle of Broth using a Syringe*

*Application to Sterility Testing*

Testing of large volume single-dose containers

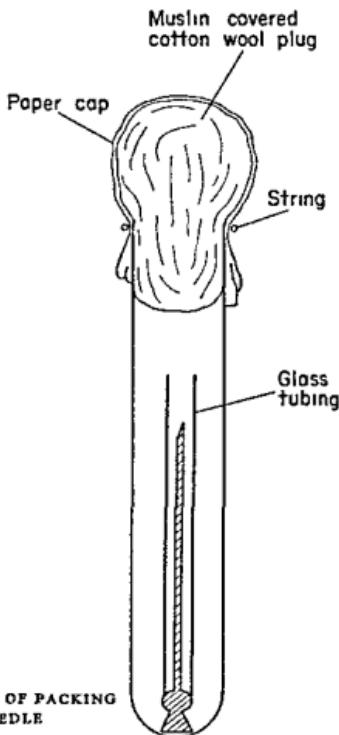
**STERILE MATERIALS REQUIRED**

6 screw-capped bottles containing 100 ml of broth, labelled 1 to 6

6 transfusion bottles with ring type closures containing 500 ml of broth and labelled 1 to 6

Six 10 ml syringes, assume that these are in glass tubes with their flanges resting on the tube rims and with the projecting parts covered with paper tied on with string

Six stout needles (e.g. 18 Gauge Serum type, 2 in long). Unless held by the shank, thin needles are difficult to get through the rubber plugs of transfusion bottles. Each needle is placed mount down in a small tube with the shank covered with a piece of glass tubing that is annealed to remove sharp edges and rests on the needle mount at the lower end (Fig 24.21). The tube is plugged and paper capped. Syringes are made with



*Fig. 24.21. METHOD OF PACKING SYRINGE NEEDLE*

two types of hub (Record and Luer), make sure that needles with the corresponding mount are supplied

Six needles, packed as above but with a cotton wool plug in each mount

#### NEW TECHNIQUES INVOLVED

Handling of transfusion bottles

Use of syringes instead of pipettes

Use of bottles, instead of tubes, of broth

*(continued overleaf)*

*Example 24 4 continued*

## PROCEDURE

1 As the 100-ml bottles are put under the screen, loosen their caps until they can be easily removed with the little finger and palm. Unlike Clinbric caps they cannot be loosened entirely or they will fall off when the bottles are picked up.

2 As the transfusion bottles are put in, swab the exposed area of the rubber plug with 75 per cent alcohol.

3 Using flamed forceps, outside the screen, remove the paper caps from the tubes of one syringe, one of the unplugged needles and all the plugged needles. Put the tubes and their contents into the screen.

4 Place one transfusion bottle in the centre of the screen, slightly towards the back. Hold the tube containing the plugged needle in the L.H. Remove the plug of the tube with the R.H., tip the tube towards the same hand and grasp the glass tubing as it falls out with the needle inside. Hold the tubing vertically so that the needle hangs within it. (Removal from the tube has thus been achieved without any danger of touching the needle shank.) Replace the plug in the tube and put it down. Transfer the tubing and needle to the L.H., take the needle mount in the R.H., remove the needle from the tubing and push it through the rubber plug of the transfusion bottle. There it will facilitate removal of the sample by admitting air (filtered through the plug in the mount) as the liquid is sucked into the syringe. Repeat with the other bottles.

5 Remove a syringe to the tile. Remove an unplugged needle from its tube by the method used for the plugged one. Transfer the needle and tubing to the L.H. Pick up the syringe in the R.H. and invert it over the needle mount, press the latter, by means of the tubing, on to the syringe hub. Revert the syringe, remove the tubing, transfer the syringe to the L.H., tighten the needle on the hub with flamed forceps and return the assembly to the R.H.

6 With the L.H. slope the bottle to the right but, because of its weight, keep it on the bench. Push the needle of the syringe through the rubber plug and withdraw 10 ml of broth. After the needle is in the bottle it should be possible to bring up the L.H. so that while the bottle is steadied with three fingers and the palm, the lower part of the syringe barrel can be held in the thumb and forefinger. Then the R.H. can be almost entirely devoted to withdrawing the plunger. Withdraw the needle from the bottle.

7 Hold a 100-ml bottle of broth near to horizontal in the L.H. Remove its cap with the R.H. Inject the broth from the syringe. Replace the cap loosely. It must not be tight because aerobic broth is used throughout these exercises and, therefore, ideal conditions for the growth of aerobes are necessary.

Before repeating the transference put the used bottles at the back of the screen, and the syringe, empty tubes and glass tubing on the unit. A lot of equipment is used for this exercise and if all of it were in the screen at once efficient working would be difficult.

It might appear easier to sample these bottles by opening them first but, in fact, little advantage is gained because—

- (i) It is not easy to remove the tight rubber plug gently.
- (ii) A sterile cover (e.g. a small beaker) must be provided for the open neck. The metal cap is no use because of the hole in the top.
- (iii) The large mouth exposed during the actual transfer increases the risk of accidental contamination and makes the use of the syringe

more difficult because it is less safe to bring the L.H. up to hold it steady

However, when the bottle is covered with a complete cap and a liner this method must be used, but the first two objections no longer apply because the closure is easily removed and can be used as a cover in the normal way

### General Advice

Most of the following points have been emphasised in the above exercises—

1 Develop methods of wrapping and packing that assist aseptic handling, e.g. the packing used for the syringe needle

2 Remove external wrappers outside the screen e.g. the paper caps of the syringe and needle tubes

3 Consider the hands contaminated and use a non touch technique as much as possible

4 Keep the tile for sterile articles

5 Do not look *under* the screen

6 Hold containers almost horizontally and caps vertically

7 Loosen screw caps before picking up the bottles

8 Develop a foolproof technique for handling and using a pipette

9 Keep the screen tidy

10 When possible perform repeated operations together, e.g. filling ampoules, removing the rubber caps of Clinbric bottles and inserting the air-escape needles into transfusion bottle plugs. However, it would be poor and even dangerous technique to remove from their tubes at the same time all the pipettes or syringes needed for a set of transferences because those not used immediately would be exposed to contamination until required

### B Aseptic Distributions

These are the techniques used in filling a number of containers with a sterile liquid from a bulk volume, e.g. an injection that has been sterilised by bacterial filtration. The essential difference between aseptic distributions and aseptic transferences is that in the former all the transferences take place from the same container, and only one piece of equipment, e.g. a

syringe is used for the lot unless there is reason to believe that it may have become contaminated. The repeated use of the same equipment and, in simple distributions (see Exercise 24.5 below), its frequent return to the bulk solution, increases the risk of contaminating both final and bulk containers.

### EXERCISE 24.5

*Distribute 1 ml of Sterile Broth into each of Six Ampoules using a Syringe*

#### STERILE MATERIALS REQUIRED

20 ml of broth in a 50 ml wide mouthed conical flask. The flask is sealed with a muslin covered plug and protected from dust by a cellulose film cap held on with an elastic band. The mouth must be wide enough to allow the syringe to pass through

A 1 ml syringe

A needle for the syringe

A tube of Durham tubes

A beaker containing eight ampoules that have been opened, annealed, washed and inverted before sterilisation. The mouth of the beaker is covered with a double layer of paper tied on with string. Unopened ampoules are unsuitable for asepsis; glass spicules produced by opening cannot be washed out aseptically, and even if this was possible, the residual water would cause volume inaccuracies.

#### PROCEDURE

1 Remove the cap from the flask. Put the latter under the screen and loosen its plug a little

(continued overleaf)

*Exercise 24.5 continued*

- 2 Put the beaker of ampoules under the screen after removing its cover
- 3 Prepare the Durham tubes (see Exercise 24.2)
- 4 Cover the ampoules and stand them on the tile or in a swabbed ampoule block.
- 5 Prepare the syringe (see Exercise 24.4) and keep it in the R.H.
- 6 Hold the flask in the L.H., remove the plug and introduce the syringe. Slowly withdraw the plunger. The volume must be measured very accurately because the contents of the final containers from a distribution will be injected. Therefore, care is taken not to draw air into the syringe because this displaces liquid from the base of the plunger. It is then difficult to decide if the correct volume has been measured. Expulsion of an air bubble with the syringe inverted should not be attempted because liquid might run down the outside of the needle, become contaminated from the air and transfer organisms to the ampoule and the bulk.
- 7 Withdraw the needle from the liquid, draw the latter further into the syringe, touch off the drop from the tip, remove the syringe and hold it inverted to prevent drops of liquid escaping due to the weight of, or accidental pressure on, the plunger. Immediately replug the flask.
- 8 Fill the first ampoule, using the method described under heat-sterilised injections, but taking particular care not to contaminate the ampoule neck when putting it over the needle because it is not possible to wipe the latter as in heat sterilisation practice. Relatively short necks are a help.
- 9 Proceed immediately to the filling of the next ampoule without putting down the syringe.
- 10 Generally, it is more convenient to seal the ampoules at another position in the room, preferably under a screen that is tall enough not to be damaged by heat from the burner. Sit meanwhile, and leave each Durham tube in position until the ampoule is on the platform.
- 11 Incubate the ampoules. They contain sufficient air for the growth of many aerobes. Also incubate the plugged flask to confirm that it has not been contaminated by repeated use.

**EXERCISE 24.6***Distribute 2.2 ml of Sterile Broth into 20 Ampoules using a Burette*

Filling with a syringe involves a considerable number of manipulations, and the probability of contaminating some of the batch increases with the number of ampoules to be filled. A method of reducing the risk is to eliminate or reduce the need to constantly refill the distribution apparatus. This is done by using a unit with a large reservoir from which many doses can be dispensed before refilling is required. When the final containers are ampoules the unit must allow very accurate measurement of the small volumes, a simple solution is to use a modified burette.

The type of burette in which the graduated tube is connected to the glass outlet by rubber tubing is most suitable for asepsis because stop-cocks are apt to seize and, particularly, leak after sterilisation. Lubrication with heat resistant silicone grease eliminates the first and reduces the second of these problems, but this solution is not perfect. Possibly P.T.F.E. stopcocks will provide the answer, when they become cheaper, because they are heat resistant and need no lubricant.

Conventional burettes require several modifications to make them suitable for aseptic filling. The glass jet must be replaced by a fine bore

hypodermic needle, with its tip ground smooth, which must be tied firmly into the tubing. Silicone rubber is preferable because it allows sterilisation by dry heat if desired. A glass hood is fitted over the needle to give protection from contamination. Preparation for sterilisation involves putting a stilette into the needle to ensure that its bore remains clear and plugging the top of the burette and its hood with muslin-covered wool. The top is also covered with two layers of paper, separately attached, and the hood is covered with one layer, (Fig. 24.22)



#### STERILE MATERIALS REQUIRED

50 ml of broth in a McCartney bottle

50 ml burette

Twenty 2-ml ampoules, opened, annealed etc and in covered beakers or a metal box

Three tubes of Durham tubes (assuming each contains about 10)

#### PROCEDURE

1 A 'fume cupboard' type screen is most convenient because the burette can be entirely inside. Shallow types must have a shielded hole in the top through which part of the burette can project. A burette stand is required, and in the 'fume cupboard' screen it must be inside. Fortunately, clean looking and easily cleaned models are available. It should be swabbed and put in during the preparation for working. A spring clip\* is also required, this should be flamed because it is used near to the filling needle. The method described is for a shallow type of screen because more precautions are necessary.

2 After removing the paper from the hood and the outer paper from the top, lower the burette through the hole and, after clamping it in the stand fix a disc of fairly thick polythene with a central hole and a radial slit over the gap left in the screen top. The disc is previously swabbed with alcohol. Put the clip on the tubing.

3 Prepare the ampoules.

4 Free the remaining paper covering the burette but do not remove it. Lift the bottle of broth up to the burette top, hold it near to horizontal, remove its cap with the L.H. and with the same hand lift out the burette plug through the paper. Using the latter as an umbrella over the mouth of the burette, carefully pour in the broth. This is a good example of the value of a double layer of paper over a plug. If only one sheet had been used it would, during storage, have collected bacteria, which would have scattered round the burette top when it was handled before and during filling. It is not difficult to fill a burette from a bottle without a funnel but if one is needed it is easy to devise a technique for using it, the reader is invited to do this.

5 Remove the plug from the burette hood, remove the stilette with forceps, and run broth down to the end of the needle. A capped specimen

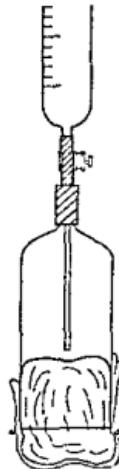


Fig. 24.22

\* Inadvertently a gate clip is shown in Fig. 24.22

**Exercise 24.6 continued**

tube is useful for collecting excess and, if necessary, for touching off the drop from the tip of the needle before filling each ampoule.

6. Carefully fill the batch. At first it may be necessary to stand to read the meniscus, but keep as still as possible and sit as soon as practicable. Seal and incubate the ampoules.

When very large batches of small ampoules or medium batches of large ones (e.g. 5 ml) are required the burette has to be filled several or many times and the problem that it was intended to solve reappears. One solution is a burette with a two-way tap that can be connected to an inverted reservoir of liquid. Alternatively, a commercial filling-machine, of which the parts that contact the liquid can be sterilised, may be used (e.g. the Fillmaster; Adelphi Manufacturing Co. N.I.).

**EXERCISE 24.7**

*Distribute approximately 20 ml of Broth into each of Six Clubritic Bottles*  
The main differences in this case are—

- The larger volume in each container. Therefore, it is desirable to replace the burette with a unit of larger capacity and to replenish this by vacuum instead of hand filling
- Slightly lower accuracy in the measurement of the volumes can be tolerated, provided that not less than the specified volume is dispensed.

**UNITS**

Units for aseptic distribution must be easy to handle; two convenient types are illustrated in Fig. 24.23. Type (a) has few rubber attachments and

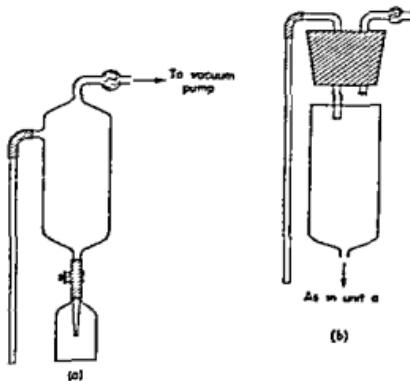


Fig. 24.23 DISTRIBUTION UNITS

can be sterilised complete but the side arms are rather easily broken. Type (b) is more robust but has several disadvantages,—

- (a) The large rubber bung, which may shed particles
- (b) The possibility of leakage points between the bung and the receiver through which organisms might be drawn when the unit is under vacuum.

- (c) If the unit is sterilised complete the bung often sticks to the receiver, therefore, it is advisable to sterilise the bung and its attachments separately
- (d) Assembly after sterilisation increases the risk of contamination
- (e) Two features require care (i) The vacuum tube must project only a short distance below the inner surface of the bung, if it is long, liquid may be sucked into the air filter and make it ineffective  
(ii) The part of the delivery tube within the unit should also be fairly short because when the vacuum is broken liquid is forced back into the bulk container until the level in the unit reaches the bottom of the tube, if the latter is long the receiver will be almost emptied

Both types of unit are fitted with fairly wide glass cannulae instead of needles, to increase the rate of filling

#### VACUUM

The most convenient arrangement is to have a small adaptor in the side or back of the screen for the attachment of vacuum tubing. The outside is connected to the vacuum point on the wall and the connexion is left permanently in position to seal the hole. The inside is connected to tubing which is long enough to reach the unit, this is sealed with a gate clip or a ball valve and is swabbed during the preparation for working, it need not be permanently attached.

#### MEASUREMENT OF VOLUMES

Possible methods are—

- (a) The receiver may be graduated. Its diameter is rather large but the slight volume errors that result are not serious in multi dose containers
- (b) The individual containers may be calibrated by standing each against a container of the same kind, containing water to the prescribed volume, and marking the required level with a glass-writing pencil. It is helpful to colour the water

#### STERILE MATERIALS REQUIRED

A distribution unit, the long arm of the delivery tube is covered with a paper cap held in position with an elastic band. The dust hoods, and the mouth of the (b) type receiver, are plugged in the usual way. A heat sealed envelope is the most convenient form of wrapping but the two parts of the (b) unit should be kept separate.

150 ml of broth in a 250 ml flask.

6 Clinbritic bottles, autoclaved, with their bakelite caps loosened to allow steam to penetrate, and afterwards dried at 60°C and then closed tightly.

12 skirted caps or rubber plugs (for Mark I and II bottles respectively) immersed in Water for Injection in a shallow screw-capped jar with a waxed card liner. The appropriate equilibrating solution would be used if an injection containing a bactericide was being distributed.

12 aluminium sealing rings, for Mark II bottles, dry heat sterilised in a small flat tin.

A pair of scissors. Cutting instruments should not be flamed because this causes oxidation, which quickly ruins their edges. Therefore, they are sterilised previously. Oxidation also takes place during prolonged

*(continued overleaf)*

*Exercise 247 continued*

heating in hot air, and the most suitable method of sterilisation is in an autoclave from which the air has been properly expelled. Autoclaves in which the steam has been produced from internal water are not ideal because the instruments are left wet and require a fairly long period of drying. The scissors are packed in a tape- or heat sealed paper envelope.

**PROCEDURE**

1 Both unit and flask need supporting, and two small clamp stands can be used. For plastic screens, plastic brackets and attachments for the back wall can be made.

2 Swab the outside of the bakelite cap of each bottle with a swab dampened with 75 per cent alcohol. There must be no likelihood of alcohol dripping into the container when the cap is removed later. Repeat with the lids of the jar of caps and the tin of rings.

3 Decap the flask and loosen its plug.

4 Unwrap the units.

*Type (a)* If in a bag, cut off the end, turn the opening towards the screen and withdraw the unit from the bag on to the tile.

If wrapped, cut the seals, turn the packet so that the freed edges are underneath, gently open the paper and, without exposing the unit, slip it from under the wrapping into the screen.

*Type (b)* First unwrap the receiver part and put it on the tile, then undo the bung, taking care to hold only the tops of the tubes. Without putting the bung down, pick up the receiver, remove the plug and put the bung firmly in place, a tight fit is essential.

5 Clamp the unit in the R.H. stand with the vacuum arm pointing to the right and the delivery tube hanging at the left. Put a gate clip on the rubber tubing in the latter. Attach to the vacuum supply. Pull the cover from the delivery arm with forceps, hold the flask near horizontal, remove its plug and then, holding the delivery tube as far from the tip as possible, insert it to the bottom of the flask. Replace the plug and clamp the flask, almost horizontally, in the L.H. stand.

6 Put a flamed, cool gate clip on the receiver outlet tube and tighten it firmly. Gently turn on the vacuum to fill the receiver, taking care to stop it in time to prevent overfilling and wetting the air filter. Close the gate clip on the delivery tube. Detach the vacuum tube, if this is forgotten the liquid will not leave the receiver when the outlet is opened.

7 Remove the plug from the hood. Hold the first bottle horizontally and, after removing its cap, turn it upright under the hood and fill.

8 Sealing the bottles

*Mark I Type*

Place the bottle in the centre of the screen with the jar of caps immediately behind.

Lift the jar lid with the L.H. and with flamed forceps pick out a cap by its skirt and tip out and gently shake off the excess water. Replace the lid.

Lift the bakelite cap of the bottle and place it on the tile. As soon as it has been removed put the rubber cap over the bottle mouth.

In the L.H. pick up a second pair of flamed forceps and use these to prevent distortion of the shank of the cap as this is pressed down into the neck with the other pair of forceps. This distortion prevents easy insertion.

The R.H. forceps should grip the whole of the flange horizontally (Fig. 24 24a). If this operation leaves the diaphragm of the cap slightly above the bottle neck it can be pressed down gently with the tips of the forceps (one of the reasons why blunt-nosed types are recommended) taking care to avoid the thin region in the centre. Excessive pressure may send the cap into the bottle. From this point there are two alternatives—

(a) *The Non touch Method* Point both pairs of forceps towards the back of the screen and grasp the left and right sides of the skirt firmly, keeping the tips of the forceps over its edge to prevent accidental perforation of the rubber. The grip must encourage the near and far edges of the

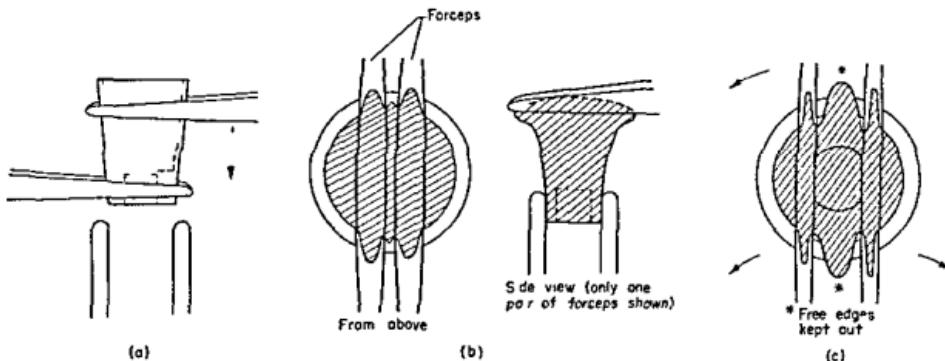


Fig. 24 24 STAGES IN PUTTING SKIRTED CAP INTO BOTTLE BY A NON TOUCH TECHNIQUE

skirt to fall outwards (Fig. 24 24b,c). Keep the forceps parallel with the bench (in particular they must not be lowered on the operator's side), turn and pull them outwards and draw the flange firmly down over the bottle neck. This method needs practise but mastery of a non-touch technique can give considerable satisfaction.

(b) *Use of One Thumb* With forceps pull the near side of the flange down over the bottle neck and hold it in position with the left thumb. Then pull the rest of the flange down with the forceps. In theory, contaminants may be transferred from the thumb to the film of moisture on the cap, and motile species may swim in this film under the flange, down between the cap and neck, and into the container. In practice there is little evidence of this occurring. If desired, sterile thumbstalls can be used, but they should be shortened to little more than a cap to facilitate aseptic insertion of the thumb.

After capping, swab the top with 75 per cent alcohol and replace the bakelite cap. The viskring can be put on after aseptic dispensing has been completed.

#### *Mark II Type*

Insert the drained plug with forceps, put on the ring and seal it with an alcohol-swabbed or dry heat sterilised closing tool. Swab with alcohol and replace the bakelite cap. Incubate the bottles.

**EXERCISE 24 8**

*Dilute a Suitable Quantity of Sterile Broth with an Equal Volume of Sterile Water and Distribute 20-ml Amounts into Six Clinbric Bottles*

This exercise is a more severe test of technique since it involves preliminary dilution of the broth

**STERILE MATERIALS REQUIRED**

Essentially as for the previous exercise, plus—

Two 100-ml glass stoppered cylinders A thin strip of greaseproof paper between the stopper and neck will prevent sticking during sterilisation The tops are paper capped

An empty, plugged 250-ml flask.

A 100 ml bottle of Water for Injection

A 100-ml bottle of double strength broth used in place of normal broth because the latter would be too weak for optimal growth of contaminants when diluted

**PROCEDURE**

1 Introduce the empty bottles, caps and rings as in the previous exercise

2 As liquid will be poured from the water and the broth bottles their necks should be flamed before they are placed inside the screen Replace the caps loosely

3 Decap the cylinders Under the screen remove the paper strips by holding each cylinder horizontally, removing the stopper and strip together (the latter should be long enough to allow this), allowing the slip to drop and replacing the stopper

4 Decap the flask and slightly loosen its plug

5 Hold a cylinder nearly horizontal in the L.H. and remove the stopper with the R.H. Hold the bottle of broth nearly horizontal in the R.H. and remove the cap with the L.H. Pour 75 ml of broth into the cylinder, keeping the latter away from vertical until the last few ml are being added A high screen is an advantage here because it facilitates eye level checking of the volume Using a similar technique, transfer the broth from the cylinder to the flask.

6 Repeat using Water for Injection and a clean cylinder Swirl the flask to mix the two liquids

7 Unwrap the unit and continue as for the previous exercise In designing this exercise two complications were introduced—

(a) The total volume required (at least 120 ml) was made greater than the capacity of the cylinder provided This made two measurements necessary If a larger cylinder had been available, or the total volume had been 100 ml or less the procedure could have been simplified by very carefully measuring the water on to the top of the broth Also, for the 100 ml cylinder, at least, it would be possible to make the delivery tube of the unit long enough to reach the bottom, thus avoiding transfer to the flask, in this case it would be useful to have available a sterile plugged cylinder from which the plug could be taken to close the mouth of the other cylinder in which the delivery tube had been inserted

(b) It was assumed that the details of the exercise were not known in time to calibrate the flask before sterilisation If this had been carefully file-marked at 75 and 150 ml the procedure would have been reduced to pouring in the liquids and mixing

**EXERCISE 24.9**

*Prepare 100 ml of a 1 per cent w/v Solution of Sterile Sodium Chloride in Sterile Broth and Distribute 15 ml into each of Six Clinbritic Bottles*

This exercise presents a new problem—the weighing of a sterile solid

A balance is required under the screen and it should be a type that can be kept clean easily (e.g. the Avery dispensing balance). A suitable time to put it into the screen is just before the tile is flamed. Swabbing shortly before use is undesirable because moisture or stickiness on any of the moving parts causes inaccuracy, and correct dosage must always be the overriding factor. Regular cleaning, occasional swabbing with 75 per cent alcohol followed, after there has been time for the alcohol to act, by careful drying with a sterile towel and storage in the asepsis room should keep the balance sufficiently clean, provided weighing is done in sterile containers of satisfactory design.

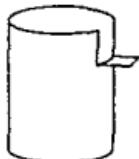


Fig. 24.25 OXOID CAP ADAPTED FOR WEIGHING

**CONTAINERS FOR WEIGHING**

Glass boats (Fig. 23.14) are suitable, particularly if the powder is directed into the covered part, but they are costly and should not be flamed. Dry heat sterilisation is used. An inexpensive alternative is an Oxoid cap that has been modified for ease of handling with forceps by making two shallow cuts in one side and bending out the part between (Fig. 24.25). This can be flamed between weighings and, if rested on its lip on the balance pan, keeps reasonably still and gives good protection to the contents.

**STERILE MATERIALS REQUIRED**

As for Exercise 24.7 plus—a wide-mouthed screw-capped bottle, with a silicone or card liner, containing sodium chloride

**PROCEDURE**

- 1 Put the bottle into the screen and loosen its cap
- 2 Pour about 90 ml of broth into the cylinder
- 3 Unwrap the boat or flame the cap. Put it on the balance pan and balance it with sterile glass beads
- 4 Hold the powder bottle horizontally, remove the cap and weigh 1 G of the powder, using a flamed spatula or spoon. Take care not to spill powder on to the pan itself
- 5 Tip the powder into the cylinder. Medicaments that do not run freely should be weighed in a boat then, after all the powder has been tipped through the stem, the remainder can be washed out by keeping the boat in the cylinder neck and gently pipetting a little broth through
- 6 Replace the stopper in the cylinder, shake to dissolve, make up to volume and distribute

**EXERCISE 24.10**

As a final stringent test Exercises 24.8 and 24.9 can be combined, e.g. Dilute sufficient sterile broth with an equal volume of sterile water and use the diluted liquid to prepare an 0.5 per cent solution of sodium citrate. Distribute 20-ml amounts into each of six Clinbritic bottles.

## REFERENCES

- 1 AEROSOLS PANEL (1949) Evaluation of aerial bactericides Report by the members of the Aerosols Panel of the British Disinfectant Manufacturer's Association *Chem & Ind* 19 Feb, 115-120
- 2 ANDERSEN, A A (1958) New sampler for the collection, sizing and enumeration of viable airborne particles *J Bact* 76, 471-484
- 3 ANNOTATION (1958) Skin antiseptics *Lancet* 2, 1164
- 4 BAKER, A H (1949) The evaluation of aerial bactericides *Chem & Ind* 18 June, 401
- 5 BATE, J (1961) The cleaning of ward floors and the bacteriological study of ward floor cleaning machines *J clin Path* 14, 32-38
- 6 BLOWERS, R and CREW, B (1960) Ventilation of operating theatres *J Hyg, Camb* 58, 427-447
- 7 BOUDILLON, R B (1948) Practical advantages of different methods of air disinfection *Medical Research Council Special Report Series No 262, Studies in Air Hygiene* Her Majesty's Stationery Office, London 311-318
- 8 BOUDILLON, R B, LIDWELL, O M and RAYMOND, W F (1948) Air sterilisation in small furnaces *ibid* 190-203
- 9 BOUDILLON, R B, LIDWELL, O M and THOMAS, J C (1948) A large slit sampler for air containing few bacteria *ibid* 19 22
- 10 BREWER, J H (1948) Aseptic operation and control of ampoule filling rooms *J Amer pharm Ass, Sci Ed* 37, 415-420
- 11 BRITISH STANDARD 2796 1956 *Preliminary assessment of aerial bactericides* British Standards Institution, London
- 12 BRITISH STANDARD 2831 1957 *Methods of test for air filters used in air conditioning and general ventilation* British Standards Institution, London
- 13 CARTER, D V (1961) Filtered air supply for sterile rooms *Hospital Pharmacy Planning* 2nd Ed. Guild of Public Pharmacists, 18, Sheepeote Road, Harrow, 23-29
- 14 CHERRY, G B, MCCANN, E P and PARKER, A (1951) The removal of bacteria from air by filtration application to industrial scale fermentations *J appl Chem* 1, S103 107
- 15 COOPER, J W and GUNN, C (1957) *Tutorial Pharmacy* 5th Ed Pitman, London 184-185
- 16 DARLOW, H M, POWELL, E O, BATE, W R and MORRIS, E J (1958) Observations on the bactericidal action of hexyl resorcinol aerosols *J Hyg, Camb* 56, 108-124
- 17 DAVIES, C N (1952) The separation of airborne dust and particles *Proc inst mech Engrs* 1B, 185-213
- 18 DE DANI, A (1955) Glass fibres, their manufacture, properties and uses *Chem & Ind* 30 April, 482-489
- 19 ELSWORTH, R, TELLING, R C and FORD, J W S (1955) Sterilisation of air by heat *J Hyg, Camb* 53, 445-457
- 20 EXTRA PHARMACOPEIA (1955) Vol II 23rd Ed. The Pharmaceutical Press, London 934-935
- 21 GUILD OF PUBLIC PHARMACISTS (1961) *Hospital Pharmacy Planning* 2nd Ed Guild of Public Pharmacists, 18, Sheepeote Road, Harrow 18-23
- 22 GUMP, W S and CADE, A R (1953) Hexachlorophene and D C M X as disinfectants for soaps *Mfg Chem* 24, 143-146
- 23 HASTINGS, J J H (1954) Problems of biochemical engineering *Trans Inst chem Engrs London* 32, 11-22
- 24 HENDERSEN, D W (1952) An apparatus for the study of air borne infection *J Hyg, Camb* 50, 53-68
- 25 HOUVINCK, E H and ROLVINK, W (1957) The quantitative assay of bacterial aerosols by electrostatic precipitation *J Hyg, Camb* 55, 544-563
- 26 HUMPHREY, A E and GADEN, E L (1955) Air sterilisation by fibrous media *Industr Engng Chem* 47, 924-930
- 27 JONTS, W N (1952) Aseptic conditions for the filling of penicillin *Pharm J* 168, 213-216, 234-236
- 28 KLUYVER, A J and VISSER, J (1950) The determination of micro-organisms in air *Antonie van Leeuwenhoek* 16, 299-310
- 29 LAWRIE, P (1952) Hexachlorophene soap effect on bacterial flora of the hands *Pharm J* 168, 288-290
- 30 LIDWELL, O M (1948) The mechanism of aerial bactericidal action *Medical Research Council Special Report Series No 262, Studies in Air Hygiene* Her Majesty's Stationery Office, London 104-122
- 31 LIDWELL, O M (1959) Impaction sampler for size grading air borne bacteria-carrying particles *J sci Instrum* 36, 3-8
- 32 LIDWELL, O M and LOVELOCK, J E (1948a) Behaviour of disinfectant vapours after dispersal in air *Medical Research Council Special Report Series No 262, Studies in Air Hygiene* H M S O London 122-130
- 33 LIDWELL, O M and LOVELOCK, J E (1948b) Simple vaporisers for aerial disinfectants *ibid* 144-147

- 34 LIDWELL, O M, LOELOCK, J E and RAYMOND, W F (1948) Propylene glycol as an air disinfectant *ibid* 75-82
- 35 LORD, J W, THOMAS, E M and PARKER, E (1952) Germicidal activity of hexachlorophene soap *Pharm J* 169, 115-116
- 36 LOELOCK, J E (1948) Aerial disinfection by substances vaporised at room temperatures *Medical Research Council Special Report Series No 262, Studies in Air Hygiene* Her Majesty's Stationery Office, London 130-139
- 37 LOWBURY, E J L (1961) Skin Disinfection *J clin Path* 14, 83-90
- 38 MACKAY, I (1952) Hexyl resorcinol as an aerial disinfectant *J Hyg Camb* 50, 82-96
- 39 McDERMOTT, W (1961) Ed Report of a conference on airborne infection *Bact Rev* 25, 173-377
- 40 MRC MEMO (1951) Medical Research Memo randum No 11 (Revised Ed) *The control of cross infection in hospitals* Her Majesty's Stationery Office, London p 49
- 41 MRC REPORT (1948) *Medical Research Council Special Report Series No 262, Studies in Air Hygiene* Her Majesty's Stationery Office London p 356
- 42 MUNDEN, D L (1952) Large scale filtration and purification of air with special reference to electrostatic precipitation *J appl Chem* 2, 65-68
- 43 NASH, T (1951) Physical aspects of air disinfection *J Hyg Camb* 49, 382-399
- 44 PRITCHARD, H (1952) The testing of disinfectant soaps *Mfg Chem* 23, 227-231.
- 45 RICHARDS, M (1955) A water-soluble filter for trapping micro-organisms *Nature, Lond* 176, 559-560
- 46 RIGBY, G L (1961) The aseptic laboratory *Chemist Drugg* 176, 120
- 47 SALLE, A J (1961) *Fundamental principles of bacteriology* 5th Ed McGraw-Hill, New York and London
- 48 SCHUSTER, E (1948) A slit sampler for prolonged recording *Medical Research Council Special Report Series No 262, Studies in Air Hygiene* Her Majesty's Stationery Office, London
- 49 SMITH, S E and WHITE, P A S (1962) Design of radioactive filtration systems *Nuclear Eng* 7, 239-245
- 50 STAIRMAND, C J (1950) Dust collection by impingement and diffusion *Trans Inst chem Engrs, London* 28, 130-139
- 51 STEEL, R (1958) *Biochemical Engineering* Heywood, London
- 52 SYKES, G (1958) *Disinfection and Sterilisation* Spon, London
- 53 SYKES, G and CARTER, D V (1953) Air filters for small aseptic units *J Pharm Pharmacol* 5, 945-953
- 54 SYKES, G and CARTER, D V (1954) The sterilisation of air *J appl Bact* 17, 286-294
- 55 TERJESEN, S G and CHERRY, G B (1947) Removal of micro-organisms from air by filtration *Trans Inst chem Engrs, London* 25, 89-96
- 56 VERDON, P E (1961) Efficiency tests on a series of skin antiseptics under ward conditions *J clin Path* 14, 91-93
- 57 WALTER, C W (1954) *The aseptic treatment of wounds* 1st Ed Macmillan, New York
- 58 WALTER, C W and ERRERA, D W (1960) *Manual supplementing the film Hospital Sepsis*, made available by Johnson & Johnson Ltd, Slough p 43
- 59 WENTE, Y A and LUCAS, R T (1956) Formation of filter materials from glass fibres *Industr Engng Chem* 48, 219-222
- 60 WHITMARSH, J M (1958) *British Fermentation Industries* Pitman, London
- 61 WILLIAMS, R E O, BLOWERS, R, GARROD, L P and SHOOTER, R A (1960) *Hospital Infection—Causes and Prevention* Lloyd-Luke, London

## 25

## Sterility Testing\*

**ASEPTICALLY PREPARED** parenteral products cannot be issued with confidence unless controls have been carried out to show that the risk of contamination from a breakdown during processing is very low. Since sterility tests are often used for this purpose it is logical to study their design, performance and interpretation before considering examples of aseptic procedures.

In the United Kingdom sterility tests are controlled by the *British Pharmacopoeia* (1963) and the Therapeutic Substances Regulations (1963) but additional advice is given in a report of a study group on General Requirements for the Sterility of Biological Substances published by the World Health Organisation (1960).

All the parenteral products of the B.P. must comply with the official sterility tests. This does not mean that samples must be tested from every batch manufactured. Occasional checks are adequate in some circumstances, such as when the preparation is sterilised by a properly controlled heating process. Nevertheless, sufficient testing must be done to provide regular confirmation that the chance of any container failing the test is negligibly small.

The following are examples of some of the preparations for which compliance with the tests is required.

**Ready-made injections**—including solutions and suspensions, both aqueous and oily

\* The revision of this book was completed before the publication of the Addendum 1964 to *The British Pharmacopoeia* 1963. This Addendum makes important changes in the Tests for Sterility which include the following:

Joint media are permitted for all tests.

Directions for batch sampling are given.

The penicillinase test for penicillins and the filtration test for other antibiotics have been modified.

Reference should be made to the Addendum for details.

**Solids for injection**—including a number of materials from biological sources, e.g. heparin, hyaluronidase and the antibiotics

#### Water for Injection

Human blood and the products obtained from it. Immunological products—vaccines, antitoxins and diagnostic preparations

Implants

Catgut

Absorbable haemostatics

This list includes oily and aqueous preparations, solids, suspensions and solutions, non inhibitory and inhibitory substances, and materials that, like catgut, are difficult to manipulate aseptically. Clearly, a considerable number of different testing techniques are required.

There is one class of sterile product—*injections sterilised by filtration*—for which the B.P. requires a test on *every* batch. Sterilisation by filtration involves quite complicated aseptic techniques, and certain faults that can arise, such as a leak in the unit or a defect in the filtering medium, are not always easily detected during the process.

The Therapeutic Substances Regulations are mainly concerned with the sterility of substances of biological origin—immunological products, the posterior pituitary hormones and insulin, antibiotics, the enzymes hyaluronidase, streptokinase and streptodornase, ligatures and sutures, and blood products. But, they also control absorbable haemostatics and a few organic chemicals—dimercaprol, curare-like compounds and certain arsenicals.

Because most therapeutic substances are very thermolabile, sterile products are obtained by filtration or aseptic technique. Consequently, except in the case of blood products, to which slightly different regulations apply, every batch of final containers must be tested for sterility and except in special

circumstances, issues are forbidden until the tests have been passed. The special circumstances are

1 When the preparation is required in an emergency by a medical practitioner and the manufacturer has no filled containers in stock. A delay of several days until the results of the tests are available would be out of the question and issue is allowed provided that—

- (a) the bulk from which the containers are filled has been tested and has passed, and
- (b) tests on samples from some of the filled containers are set up, examined daily and, if contamination is detected, the practitioner is notified at once

2 When the substance is so unstable that it loses appreciable activity if held until completion of the tests. In this case the bulk test is waived and only (b) applies. Liquid B.C.G. vaccine, now largely replaced by the more stable freeze-dried form, is an example

The pharmacopoeial medicaments and preparations that are therapeutic substances must also comply with the Therapeutic Substances Regulations when these are more stringent than the requirements of the B.P.

#### INFORMATION GIVEN BY A STERILITY TEST

Sterility, in the microbiological sense means freedom from living micro organisms and, therefore, it is not possible to claim that a batch of products is sterile unless—

- (a) the entire content of every container in the batch has been tested, and

Important aspects of sampling are

#### 1 STAGES AT WHICH SAMPLES SHOULD BE TAKEN

The need for tests on the final containers is incontrovertible and, for heat sterilised products this is the only stage at which they are relevant. However, when processing involves aseptic technique it is advisable to test, also, the bulk from which the final containers will be filled. For example, products sterilised by filtration can be contaminated during filtration or filling, by performing bulk as well as final container tests it is possible to discover which of these stages is responsible. Also, as explained earlier, emergency issue of containers is allowed if the bulk has been tested.

[The World Health Organisation (WHO) Report

- (b) the test provides optimum conditions for the growth and multiplication of every organism, vegetative or spore, healthy or injured, that might be a contaminant

Unfortunately, neither of these conditions can be satisfied because—

- (a) in sterility testing the article or preparation under test is either destroyed (e.g. an injection solution) or made unusable (e.g. a syringe), therefore, only part of the batch can be sampled,
- (b) even when great care is taken to provide media and incubation conditions satisfactory for most organisms it is impossible to supply all the variations necessary to ensure that every type and condition of contaminant will grow

Consequently, sterility tests can only show that organisms capable of growing in the test media under the selected conditions are absent from the fraction of the batch that has been tested. Two conclusions can be drawn from this

1 Sterility testing should not be used as the sole means of controlling sterile processing. Heat sterilisation methods can be checked instrumentally and bacteriologically (see p. 343) and procedures involving asepsis may be controlled by careful supervision of operatives, regular air sampling (within and outside the screen) and full scale runs using nutrient broth.

2 To obtain reliable results from sterility tests it is necessary to take sufficient samples, to use sensitive culture media and during testing, to reduce accidental contamination to a minimum.

#### Sampling

recommends bulk and final container tests, the Therapeutic Substances Regulations do not require the former except in connexion with emergency issues, and the tests of the British, United States and International Pharmacopoeias refer to final containers only.]

#### 2 SELECTION OF THE SAMPLES

Samples must be representative of the whole of the bulk material and the lot of final containers.

*For the Bulk*, the material must be thoroughly mixed before the sample is taken.

*For Final Containers*, the samples must be selected at random, but—

- (a) When a load from a heat sterilisation process is being tested samples should be taken from every

shelf and from any parts of the steriliser in which less satisfactory sterilising conditions are believed to exist. See, for example, the directions in the *United States Pharmacopoeia* for products sterilised by steam under pressure.

(b) For aseptically-processed preparations samples must be taken throughout the filling operation. The *United States Pharmacopoeia* defines the latter as a period in which there is no change in the filling assembly or equipment and which is not longer than a day.

### 3 SAMPLE SIZE

From the Bulk, the amount taken must include sufficient for repeat tests in case these are necessary.

The WHO Report recommends the inoculation of at least 5 ml into one or more vessels of each culture medium at each temperature of incubation. The Therapeutic Substances Regulations require at least 0.1 per cent (with a minimum of 1 ml) if the volume is less than 10 litres, and at least 10 ml if the volume is more than 10 litres.]

From the Final Containers The guiding principle is stated very clearly in the WHO Report 'The number taken should reduce to a minimum the risk of releasing contaminated containers' Bryce (1956) and Knudsen (1949), using sampling statistics, have shown that contaminated batches may easily be passed as sterile. For example—

(a) Knudsen calculated that if a lot contains 5 per cent of contaminated containers the probability of failing to detect the contamination is 60, 45 and 35 per cent respectively when 10, 15 and 20 containers are tested.

(b) Bryce, expressing the same results in another way, points out that if 6.5 per cent of a lot are contaminated there is a 1 in 4 chance of selecting 20 consecutive sterile containers and, therefore, of passing the lot. If only 10 containers are sampled the risk of accepting the same lot rises to 1 in 2, i.e. 50 per cent.

Most countries use one of two sampling rules—

- (i) A fixed percentage of the final containers is selected

(ii) A fixed number of containers is taken, independent of the lot size.

At first sight the former method might appear the most logical but in fact, as Knudsen has shown, it results in greater leniency with small lots. This is because information about the contamination of the lot is obtained from the sample batch and, if this is small, as it will be when a small lot is sampled on a percentage basis, the resulting information is smaller and, consequently, less accurate. Savage (1961), in a slightly different context, gives an example that can be used to illustrate this point, if the sample size is 2 per cent and the lot under test consists of 500 containers of which 1 in every 10 is infected, the number tested will be 10 and, since one of these will almost certainly give a positive result, the whole batch will be rejected. If, however, the same number of containers (with the same percentage contamination) had been prepared in lots of 50 on 10 separate days, only 1 container (2 per cent of 50) from each would be tested and, as a result, 9 out of 10 of the batches would almost certainly pass. This would not occur if the same number of containers was used independent of the lot size.

The WHO Study Group could not decide between the two rules and suggested that, for the present, any rule based on sampling statistics and found acceptable by a national controlling authority should be regarded as acceptable. It also recommended for consideration an alternative rule, intermediate between the others, in which the sample is  $0.4\sqrt{N}$ , where  $N$  is the number of containers in the final lot.

[The B.P. and I.P. have no sampling rule. The Therapeutic Substances Regulations require the testing of 2 per cent or 20 (whichever is smaller) but for lots of over 1,000 an additional 2 containers for each succeeding thousand or part of a thousand is considered adequate. The requirements of the U.S.P. for products sterilised by steam under pressure are different from those for other products. For the former 10 or more units must be taken from each load, and for the latter, because the risk of contamination is greater, 20 or more units are specified.]

(See also Davies and Fishburn, 1948, Kelsey, 1961, Proom, 1962 and Tattersall, 1961)

### Culture

#### A SENSITIVITY

The importance of using sensitive media in sterility testing was explained in chapter 19. The following requirements of the *British Pharmacopoeia* are similar to those of other authorities concerned with sterility testing.

### Media

Sterility test media must—

Initiate and maintain the vigorous growth of Small numbers of the Aerobic and anaerobic bacteria that can be cultivated on artificial culture media including Common saprophytes

**Pyogenic cocci** (the WHO Report mentions *Staphylococcus aureus* and *Streptococcus pyogenes*, Group A) and

Spore-bearing bacteria pathogenic to man

Cook (1961) comments on some of the problems of confirming these abilities. He mentions the lack of information on which bacteria are common contaminants and the difficulty of defining a small number of organisms. He emphasises the importance of qualitative, in addition to quantitative, tests because although a medium may initiate and support the growth of a very small inoculum of a particular organism it may be much less sensitive for other species (see also Sykes, 1956). He refers also to the different growth requirements of healthy cells and bacteria that have been damaged but not killed by physical and chemical agents (see also Jacobs and Harris, 1961).

Sykes (1956) points out that although some fastidious pathogens (e.g. *Diplococcus pneumoniae*) may not grow readily in the usual test media, and others (e.g. *Mycobacterium tuberculosis*) will multiply too slowly for detection in the normal incubation period, preparations contaminated with these are unlikely to be passed as sterile because they will almost certainly contain other contaminants that can grow in the media.

#### B TYPES

Of the previously mentioned sources on sterility testing the B.P. and I.P. are exceptional in not appearing to encourage the use of a single medium capable of supporting the growth of both aerobes and anaerobes. The only reference in the B.P. is in connexion with the filtration technique for antibiotics. The WHO Report recommends the U.S.P. uses, and the Therapeutic Substances Regulations allow such a medium.

In these joint media, although conditions at the bottom are suitable for strict anaerobes, there is enough oxygen at the top for the growth of strict aerobes while, in between, is a gradient of oxygen concentration in which organisms with intermediate requirements can find a suitable environment.

Preference for separate aerobic and anaerobic media is based on the assumption that media specifically designed for each of the two classes of organism are likely to be more sensitive than any single medium that attempts to satisfy the needs of both.

The WHO Report lists a number of media used for sterility testing in various parts of the world but emphasises that others might be equally suitable. It classifies them as follows:

#### 1 For Detection of Aerobes

- (a) *Peptone Broth* This is a name often used for meat extract broths containing peptone.
- (b) *Glucose Peptone Broth* This differs from (a) only in containing 0.5 per cent of dextrose, which promotes luxuriant growth of many organisms.

Digest broths would be acceptable alternatives to these two media and are particularly valuable for organisms with exacting growth requirements.

#### 2 For Detection of Anaerobes

- (a) *Cooked meat Medium*
- (b) *Senni fluid Meat Medium* (Sloppy agar)
- (c) *Lüer Broth* This is often used in the examination of foods for anaerobes.

Although primarily recommended for anaerobes, these media contain sufficient oxygen in the upper regions to permit the growth of aerobes as well.

#### 3 For Detection of Aerobes and Anaerobes

Seven media are listed in this category. In the majority a satisfactory oxidation reduction potential for anaerobic growth is produced by sodium thioglycollate, sodium hydrosulphite or both. The most important are—

(a) *Fluid Thioglycollate Medium* This is Linden's modification of Brewer's original thioglycollate medium, further modified by Pittman (1946). It is one of the formulae recommended in the U.S.P. and contains the following ingredients—

Reducing agents—sodium thioglycollate and dextrose

A substance to increase viscosity—agar

An oxidation reduction indicator—resazurin

Nutrients—pancreatic digest of casein (nitrogen source)

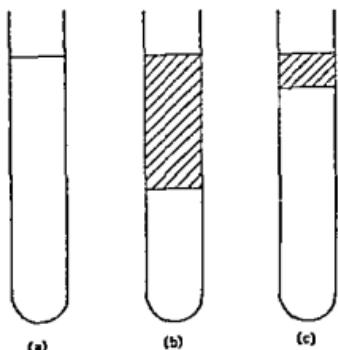
—dextrose (carbon source)

—yeast extract (growth factor source)

—sodium chloride

The amino acid L-cystine is also included because it encourages the growth of certain Clostridia (Pittman 1946).

This formula is suitable for the detection of anaerobes if not more than the upper 30 per cent is coloured (Fig. 251), for other thioglycollate formulae 20 per cent is usually regarded as the limit. If necessary, reduced conditions may be restored by heating in a water bath until the pink colour disappears, this treatment must not be repeated because



*Fig. 251 THIOLYCOLATE MEDIUM IN VARIOUS STATES OF REDUCTION*

- (a) Completely reduced—ideal as an anaerobic medium
- (b) More than 50 per cent coloured—unsuitable as an anaerobic medium
- (c) Small coloured ring—desirable when used as a joint medium

frequent reheating gives rise to toxic degradation products. A medium in the condition, shown in Fig. 251c, is more suitable than a completely reduced medium because aerobic growth will be more quickly initiated.

(For further information see the Disco Manual, 1953.)

(b) *Thioglycollate Broth Medium*. This is the alter native medium of the U.S.P. It differs from (a) in containing no agar or indicator.

It is used when the high viscosity of (a) prevents satisfactory dispersal of certain test materials throughout the medium. Preparations difficult to

distribute include semi-solids, such as creams, and turbid preparations, e.g. suspensions.

Because it contains no agar to retard inward diffusion of oxygen this medium must be heated, as above, not more than 4 hours before use.

(c) *Corn Steep Liquor-Sodium Thioglycollate Medium*. This differs from (a) in the following respects—

- (i) The nitrogen source is meat extract instead of casein digest.
- (ii) The growth factor source is corn steep liquor instead of yeast extract.
- (iii) It contains sodium hydrosulphite as an additional reducing agent.
- (iv) There is no special supplement of L-cystine.

(d) *Semi-fluid Hydrosulphite Medium*. This is Clausen's modification of a medium developed by Bonnel. The differences from (a) are—

- (i) Sodium hydrosulphite replaces sodium thioglycollate as the main reducing agent.
- (ii) Peptone replaces the casein digest.
- (iii) The agar concentration is almost twice as large.
- (iv) No L-cystine supplement.

Ježkova (1960) compared the sensitivities of several sterility test media including the fluid thioglycollate, semi fluid hydrosulfite and liver broths. Using 272 strains from 20 species, but including only one anaerobe, this worker found the fluid thioglycollate to be the most sensitive at 32°C, and equal best with the hydrosulphite medium at 37°C. None of the media was absolutely satisfactory thus confirming the statement made earlier in this chapter that no sterility test medium can be more than a compromise.

#### Precautions Against Accidental Contamination

The primary aim of sterility testing is to prevent the issue of contaminated materials but it is also important to ensure that there is little risk of rejecting a sterile batch because its destruction or resterilisation, where the latter is practicable, can involve considerable financial loss. Therefore, strict precautions must be taken to minimise accidental contamination during testing. For example—

1 Tests should be performed under a screen in a properly ventilated asepsis room supplied with bacteriologically clean air. Ultra violet light or aerosols should not be used because they might sterilise contaminated articles under test and, in the case of aerosols, cause inhibition in the test media.

2 Staff must fully appreciate the need for rigorous aseptic technique and be given expert training in-

cluding exercises similar to those described in the previous chapter, before they are allowed to carry out tests.

3 Adequate control tests must be regularly performed (see below).

Royce and Sykes (1955) developed a method of testing that does not require an asepsis room, special clothing or elaborate preparation for working. It involves the use of a glove box (an entirely enclosed screen fitted with rubber gauntlets) in which the outer sides of sample and media containers and the sampling equipment are sterilised by overnight treatment with ethylene oxide. It can be used only for materials in containers entirely impermeable to the gas. A more detailed description is given in chapter 27.

### Testing Procedure

Prescribed quantities from the bulk or from each container of the material under test are transferred to suitable volumes of medium and, after incubation at specified temperatures, are examined for signs of living organisms

#### 1 PRESCRIBED QUANTITIES

Bulk sampling volumes have been given earlier. Volumes from final containers differ with different authorities, several examples are listed below to illustrate the variety of ways in which the problem has been tackled—

##### (a) For Solutions and Suspensions

*British and International pharmacopoeias* If the volume in the container is less than 2 ml, half is used for a test for aerobes and half for a test for anaerobes. If the volume is 2 ml or more, 1 ml is taken for each test.

*Therapeutic Substances Regulations* As above, except that a single medium in which both aerobes and anaerobes can be detected is permitted as an alternative to separate aerobic and anaerobic media. If the single medium is chosen, twice the sample volume used in the separate media must be added.

##### United States Pharmacopoeia

Container volume	Minimum sample volume	Minimum medium volume
Less than 1 ml	All	15 ml
10 ml or less	1 ml	15 ml
10 to 50 ml	5 ml	40 ml
More than 50 ml	10 ml	40 ml

A medium that supports aerobes and anaerobes is used and minimum volumes are specified to ensure that the sample does not cause excessive dilution of the ingredients and so impair the growth-promoting properties of the broth.

*WHO Report* At least 1 ml or at least half of the human dose, whichever is larger, with an upper limit of 10 ml. The importance of relating medium volume to sample size is mentioned.

##### (b) For Powders

*British and International pharmacopoeias* If the weight in the container is less than 100 mg, half is used for a test for aerobes and half for a test for anaerobes. If the weight is 100 mg or more, 50 mg is taken for each test.

*Therapeutic Substances Regulations* As above, with the modifications given under solutions and suspensions.

*United States Pharmacopoeia* 300 mg or, if the weight in the container is less than 300 mg, the entire contents are added to 40 ml of the medium.

*WHO Report* At least 25 mg or at least half the human dose, whichever is larger, with an upper limit of 500 mg.

#### 2 INCUBATION

##### (a) Temperature

The Therapeutic Substances Regulations recommend 37°C but, as explained in chapter 19, this is not entirely satisfactory because some pathogens will not grow well at this temperature. Therefore, the B.P. and U.S.P. specify 30°–32°C. The WHO Report suggests either (i) the U.S.P. temperature or (ii) the setting up of two tests and the incubation of one at 35°–37°C and the other at 15°–22°C. The latter will assist the detection of *Pseudomonas spp.*, the psychrotrophs that damage blood and other nutritive products in cold storage, and saprophytic accidental contaminants.

##### (b) Time

The Therapeutic Substances Regulations specify 5 days, the B.P. recommends 7, and the U.S.P. and WHO Report suggest 7 or more. The aim is to provide an adequate period for the recovery of organisms suffering from the effects of heat or antibacterial agents and for the multiplication of these, and the normally small numbers of other contaminants, into a detectable turbidity.

##### (c) Frequency of Inspection

The containers should be inspected often, e.g. every day, because some bacteria produce a detectable turbidity at first, which later settles as an insignificant deposit at the bottom of the tube and leaves clear, apparently uncontaminated broth above. It is always advisable to swirl containers gently before examination to stir up any sediment. Another advantage of frequent inspections is that repeat tests (where these are permitted) can be set up immediately contamination is detected.

For further information on these three aspects of incubation see Sykes (1956).

#### 3 INTERPRETATION OF THE RESULTS

The sample and, therefore, the bulk or lot passes the test if there is no sign of growth in any of the containers.

If growth is obtained, all the previously mentioned authorities allow the test to be repeated with fresh

samples. This is an attempt to rule out the possibility that contamination in the first test came from faulty technique. Repeat tests should be carried out with exceptional care to prevent a recurrence of accidental contamination.

If growth occurs in the second test the *British Pharmacopœia* and Therapeutic Substances Regulations permit a further repeat unless the same organism is found in the two tests. In the latter case the sample fails because it is assumed that the product must be heavily contaminated if the same organism can be recovered from two randomly selected batches of samples.

If the third test is negative the sample passes, and if it is positive the sample fails.

The WHO Report recommends only one repeat of tests on bulk material, here the risk of accidental contamination is lower because fewer manipulations are involved than when final containers are tested. It allows two repeats of final container tests but the batch fails if there are three or more, two or more and any positive results in the first, second and third tests respectively. The *United States Pharmacopœia* does not allow the third test and requires doubling of the samples in the second.

Repeat tests have been strongly criticised. Cook (1961) points out that if contamination is due to—

(a) *The Sample* Retesting increases the likelihood of passing a contaminated batch. For example, if repeats are not allowed a sample batch of, say 10

containers, will fail if one gives a positive result, but, if a repeat is allowed and all the results are negative the contaminated lot will pass in spite of the positive in the first series. Davies and Fishburn (1948) calculated the chances of passing infected lots when sample batches of ten are used, they made the assumption that only one type of contaminant was present and, therefore, that the lot would fail if the test and first repeat were positive. As an example of their results, if the lot contains 10 per cent of infected containers there is more than an even chance (actually 58 per cent) of passing it. If the contaminants in the test and repeat are different the chance is still further increased because another repeat is allowed. Savage's example (p. 448) further illustrated this point. The one batch of 50 ampoules that failed would be retested and, since only one ampoule would be used and only 10 per cent of the batch are contaminated, would almost certainly pass.

(b) *Accidental Contamination* It is not improbable that the repeat test will become contaminated from the same source and, therefore, the aim, of preventing the rejection of a sterile lot, will not be assisted.

Finally, a quotation from Bryce (1956) referring to the interpretation of the repeat tests of the B.P. and Therapeutic Substances Regulations 'a batch, to consider an extreme case, could turn up anthrax (in the first test) and tetanus (in the second) and yet be regarded as having passed.'

#### Control Tests

The results of sterility tests cannot be relied upon if they can have any other explanation except—

- 1 In the case of a negative result—sterility of the sample
- 2 In the case of a positive result—contamination of the sample

However, a negative result could also be due to—

(a) *Inability of the Broth to Support Bacterial Growth* Possible causes of this are—

- (i) Inadequate formulation
- (ii) Accidental omission of an ingredient
- (iii) Overheating during preparation and sterilisation
- (iv) In anaerobic media, failure to boil off oxygen from excessively oxygenated containers

(b) *Inhibition of Contaminants by a Substance added in the Test* This could be—

- (i) The sample itself

(ii) A neutralising agent used to destroy the antibacterial effect of an ingredient of the sample

Similarly, a positive result could be caused by—

- (a) *Lack of sterility of the medium*
- (b) *Accidental contamination during testing*

Therefore, control tests are essential to show that these factors are not or are most unlikely to be the explanation of the result.

#### I NEGATIVE CONTROLS

In these no growth is expected.

(a) A container of medium from each batch used for the test is incubated at the same time as the test containers

This control serves three purposes—

- (i) It confirms that the medium is sterile
- (ii) It shows that the oxidation reduction qualities of indicator-containing anaerobic media are

satisfactory If they are not, the colour quickly spreads down from the surface of the medium  
 (iii) It serves as a standard with which the corresponding test container can be compared during and after incubation A faint turbidity is more easily detected if the suspect tube is examined at the side of the control This method is particularly helpful with anaerobic media since these are not always perfectly clear and, in the absence of a standard a sterile tube, might be interpreted as contaminated

(b) Any substance, other than the sample, added to a test-tube should be proved sterile by incubating suitable amounts in appropriate media An example is penicillinase, a solution of which is used in testing penicillin

## 2 POSITIVE CONTROLS

In these growth is expected

(a) The sensitivity of the media must be confirmed Although this is done in connexion with the formulation and selection of media for sterility testing, at the time of use it is desirable to check that the sensitivity has not been affected by any error in preparation or storage

An exacting aerobe and an exacting anaerobe are inoculated into containers of the appropriate media and examined for growth after a short time interval Small inocula are used, e.g. 1 ml volumes of 1 in 100,000 dilutions of 18-24 hr cultures grown in 10 ml quantities of broth

When a medium capable of detecting aerobes and anaerobes is used for the test the two types of organisms should be added to separate control containers because if they are inoculated into the same one it may not be possible to decide whether both have grown or not

### Inactivation of Antibacterial Samples

Many of the pharmaceuticals that must be tested for sterility are, or contain, medicaments that can either destroy bacteria or prevent their growth Others are preparations in which a bactericide has been included (e.g. multidose injections and injections sterilised by Heating with a Bactericide) When samples from these are added to test media the concentration of the antibacterial agent may be sufficient to prevent bacterial growth and, therefore, the detection of contaminants

It might be thought that these organisms could be ignored since they are so successfully inhibited by the product, but animal experiments have shown that bacteria inhibited by certain antibacterials can be-

(b) The medium must be shown capable of supporting the growth of small numbers of bacteria in the presence of the sample

This is the most important control and must never be omitted

Organisms, as above, are added to containers in which there are exactly the same quantities of the same inclusions as in the tests

## 3 CONTROLS TO CHECK WORKING CONDITIONS AND OPERATORS' TECHNIQUE

These are used at regular and frequent intervals to confirm that the risk of accidental contamination is low Examples are—

(a) *General Air Sampling* This shows that the high quality of the air supply to the room is being maintained

(b) *Air Sampling at Each Working Space* Settling plates under and near the screen help to detect poor technique and, particularly, excessive movement

(c) *'Dummy Runs'*. Tests are performed with materials known to be sterile, e.g. ampoules or bottles of Water for Injection or sodium chloride that have been sterilised for longer times and/or higher temperatures than normal Preferably, operators should not be aware that the run is a control because this might encourage exceptional care, for this reason broth, which is easily recognised, is not an ideal liquid to use

### INOCULATION OF POSITIVE CONTROLS

Since the reason for carrying out sterility tests in an asepsis room and with good aseptic technique is to reduce the risk of accidental contamination during testing, the use of micro organisms in the same laboratory is most undesirable Controls should be inoculated in a separate bacteriology laboratory

come revived after injection (see Klarmann, 1950), presumably because the inhibitor is diluted by the body fluids or neutralised by chemicals in the tissues Consequently, it is important to detect inhibited micro-organisms A further reason is that the presence of living bacteria indicates the breakdown of a sterilisation or aseptic process which will not be detected if the organisms cannot be grown

The methods used most frequently are—

1 The sample is added to a volume of medium sufficient to dilute the inhibitor to below its minimum bacteriostatic concentration

2 When dilution is impracticable, the sample is

treated with a substance capable of neutralising the inhibitor, the neutraliser must not be antibacterial itself.

3 When neither dilution nor neutralisation is possible, contaminating organisms are separated from the sample by filtration through a bacteriaproof membrane and, after washing free from inhibitor, transferred on the membrane to a suitable container of culture medium.

### A INACTIVATION BY DILUTION

The relationship between the concentration of a bactericide and the rate at which it kills bacteria is given by the expression

$$C^n t = \text{a constant.}$$

where  $C$  is the concentration of the bactericide,  $t$  is the time taken to kill the bacteria, and  $n$  is the dilution coefficient. The dilution coefficient indicates the effect of dilution on the rate of bactericidal action. For example, assuming that a substance has a dilution coefficient of 6 and that a 1 per cent solution kills a certain population of bacteria in 10 minutes, then, if the concentration is reduced to 0.5 per cent the time in which the same population will be killed can be calculated from the expression

$$\begin{aligned} C_1^n t_1 &= C_2^n t_2 \\ \text{i.e. } 1^6 \times 10 &= (\frac{1}{2})^6 \times t_2 \\ 10 &= \frac{1}{64} t_2 \\ t_2 &= 640 \text{ min} \end{aligned}$$

i.e. the activity of the substance has been greatly reduced.

Consequently, the antibacterial activity of compounds with high dilution coefficients can be eliminated fairly easily in sterility testing by adding the sample to a volume of broth that, although larger than usual, is still reasonably small. For example—

#### 1. Phenolic Substances

The phenolic compounds used as bactericides in injections (e.g. phenol, cresol, chlorocresol and the esters of parahydroxybenzoic acid) can be inactivated by adding 1 ml of the sample to 50 ml of the culture medium. Although in some cases, such as chlorocresol and the hydroxybenzoates, this dilution is well in excess of the minimum necessary, it is convenient to use the same procedure for the whole group of compounds because this dispenses with the need to pack a variety of volumes of broth between 10, the normal quantity when no inhibitor is present, and 50 ml.

Dilution must be carried out in the final test medium. If the preparation is diluted with, say, sterile water, and 1 ml of the dilution is used as the inoculum, the sensitivity of the test is reduced because only  $\frac{1}{6}$  of the specified sample volume will be added to the broth. A possible result is illustrated by the following example.

If the original preparation contained 1,000 organisms in 10 ml, a 1 ml sample will contain 100.

But 1 ml of a 1 to 50 dilution of the preparation will contain only 2 and, therefore, contamination of the preparation may not be detected because so few organisms are transferred to the culture medium (see the section on 'The Growth Curve' in chapter 19).

Pre-dilution also introduces additional manipulations and an extra material (the water, above), each of these might be a source of contamination.

#### 2. Alcohols

A 1 to 50 dilution in the test medium is also suitable for the bactericides chlorbutol and benzyl alcohol.

#### 3 Barbiturates

Booth (1955) has shown that in concentrations greater than about 0.2 per cent the sodium salts of barbitone, hexobarbitone, phenobarbitone and thiopentone may prevent bacterial growth. This is due to the high pH (in excess of 9 in some cases) of their solutions and, possibly, to inhibition by the drugs themselves.

These substances are usually tested for sterility in the dry state because the injections are prepared immediately before use. The 50 mg sample directed in the B.P. should be added to 50 ml of broth to ensure adequate dilution.

### B INACTIVATION BY NEUTRALISATION

Inactivation by dilution is impracticable in two circumstances—

(a) When the antibacterial agent has a low dilution coefficient.

If this is 1 and, as in the example given above, a 1 per cent solution kills a bacterial population in 10 minutes, then, when the concentration is reduced to 0.5 per cent the effect on the activity of the substance is relatively small.

$$\begin{aligned} C_1^n t_1 &= C_2^n t_2 \\ \text{i.e. } 1^1 \times 10 &= (\frac{1}{2})^1 \times t_2 \\ t_2 &= 20 \text{ min.} \end{aligned}$$

Consequently, very great dilutions are necessary in sterility testing to remove the effects of this type of

compound. Generally, they are impracticable because large volumes of broth are costly and difficult to manipulate, and the test is less sensitive if the sample size is small relative to the volume of medium.

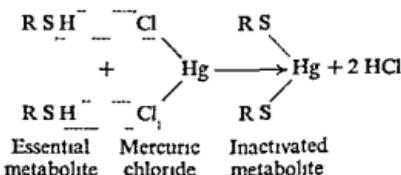
(b) When the inhibitor is strongly adsorbed by, or combined with, the bacterial cell wall

If this occurs, as, for example, with organic mercurials and quaternary ammonium compounds, even very high dilution will not prevent continued inhibition of the organisms after transfer to the test medium.

In these cases, the effect of the antibacterial agent can sometimes be overcome by chemical neutralisation—

### 1. Mercurials

Mercurial antibacterial agents, such as mercuric chloride, phenylmercuric salts and thiomersal are believed to inhibit bacteria by combining with the sulphhydryl ( $-SH$ ) groups of vital compounds, e.g.



As long ago as 1889, Geppert discovered that organisms apparently dead from exposure to mercuric chloride could be revived by treatment with ammonium sulphide, the mercury being removed from the metabolite in the form of insoluble sulphide. This method cannot be used for organic mercurial compounds because the mercury is part of a complex ion and will not react with the sulphide. However, all mercurials have less affinity for bacterial metabolites than for certain other sulphhydryl compounds and will leave the former in preference for the latter with consequent revival of inhibited cells. One of the most efficient revivers is thioglycollate (Cook and Steel, 1960, Steel, 1960) and the amount (0.05 per cent) present in the thioglycollate media recommended for sterility testing is sufficient to neutralise the mercurials in the prescribed volumes of injection solutions if a sufficient quantity of medium is used.

### 2. Arsenicals

The antimicrobial arsenic compounds (e.g. neoarsphenamine, sulpharsphenamine, tryparsamide and the oxaphenarsine salts) are believed to act in the same way as the mercurials and, therefore, their in-

hibitory effects can be similarly neutralised—with sulphhydryl compounds.

Sykes, Royce and Hugo (1952) investigated the sterility testing of neo- and sulpharsphenamine and recommended—

- (a) Limitation of the concentration of the arsenical in the test medium to not more than 0.5 per cent
- (b) The use of a tryptic digest medium containing—

(i) 0.4 per cent sodium thioglycollate. The high concentration is needed because of the comparatively large amount of arsenical that is added in the test-up to 0.5 per cent (Compare the amount of phenylmercuric nitrate (0.002 per cent before dilution in the medium) that would be added in a sample from an injection containing this substance as a bactericide.)

(ii) A 1-cm depth of cooked meat at the bottom. This contains natural thiol (sulphhydryl) compounds which supplement the sodium thioglycollate and provide sufficient neutraliser to overcome fully the inhibitory effect of the arsenical. The thioglycollate concentration cannot be increased further, to avoid addition of meat, because it is inhibitory at about 0.5 per cent.

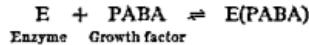
(iii) Subculture after incubation. Organic arsenicals produce cloudiness, due to reaction with ingredients in the medium, and this must be distinguished from bacterial growth (see 'Tests on Insoluble Powders').

(See also Berry and Jensen, 1951)

### 3. Sulphonamides

Although the evidence is not incontrovertible, it is generally assumed that the sulphonamides inhibit bacterial multiplication by interfering with the utilisation of the important growth factor para amino-benzoic acid (PABA).

If

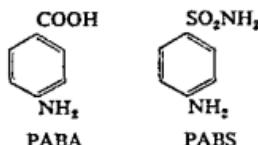


is an essential step in the metabolism of an organism and an antibacterial agent (in this case a sulphonamide, e.g. para aminobenzenesulphonamide—PABS) can also combine with E, viz.—



Adequate amounts of E(PABA) will not be produced, the metabolic chain will be broken and the organism will be unable to grow or multiply. This type of antibacterial action is known as competitive inhibition. The structural similarity between PABA and

the parent sulphonamide, PABS, explains why sulphonamides can compete with PABA for combining sites on the enzyme



However, sulphonamide inhibition is overcome if the environment contains sufficient PABA. This fact is used in the sterility testing of preparations containing sulphonamides, PABA being added to the broth to antagonise the inhibitor and revive the contaminants. A low concentration is sufficient because the enzyme has a much greater affinity for the growth factor than for the antagonist, e.g. 1 molecule of the acid antagonises about 5,000 molecules of PABS. Usually, 5 or 10 mg/100 ml is added during the preparation of the broth (aerobic and anaerobic or joint) which is then called PABA broth. Sykes (1956) states that, in practice, 250 ml of an 0.01 per cent solution in broth is sufficient to neutralise up to 5 G of sulphanilamide (PABS) or sulphathiazole.

#### 4 Quaternary Ammonium Compounds

These substances, examples of which are cetyltrimide, benzalkonium chloride and domiphen bromide owe their detergent and antibacterial properties to their complex cations. They are bacteriostatic in very high dilutions and, because of their great surface activities, are strongly adsorbed on to the cell walls of micro-organisms. Consequently, it is not possible to use dilution to overcome their effects in sterility testing and an antagonist is essential. Many have been investigated, and the most obvious are anionic detergents such as soaps and sodium lauryl sulphate, the large anions of these combine with and precipitate the active cations of the inhibitors, they are unsuitable for use in sterility testing because they have bacteriostatic activity themselves and high concentrations are necessary for full neutralisation.

One of the best antagonists is a mixture of lecithin and a non-ionic surface active compound. Suggested modes of action of the lecithin are production of insoluble or feebly ionised complexes with the quaternaries or alteration of the cell membrane (of which lecithin is an important component) making it less permeable (Klarman, 1950). The neutralising effect of certain non-ionic surfactants may be the result of chemical bonding with the inhibitor (Delaia and

Kostenbauder, 1960). This mixture can be used in two ways

(a) *In the Medium*. An example is Lethen broth (Quisno, Gibby and Foter, 1946) which contains 0.07 per cent of egg lecithin and 0.5 per cent of Tween 80 (a non-ionic surfactant, it is a polyoxyethylene ether of sorbitan mono-oletate). Originally the Tween was added to solubilise and disperse the lecithin. Quisno *et al* found that 10 ml of this medium would neutralise 1 ml of a 1:1,500 dilution of several quaternary ammonium compounds.

(b) *Before Transfer to the Medium*. In this case higher concentrations of the neutralisers can be used, e.g. 2 per cent of lecithin and 3 per cent of the non-ionic compound (B.S. 3286 1960 gives further details of this and other neutralising solutions).

*See also* Allport (1952) and Lawrence (1948, 1950).

#### 5 Penicillin

In earlier chapters references have been made to the enzyme penicillinase. This is produced by a wide range of bacteria but, fortunately, not by many common pathogens. It inactivates penicillin by hydrolysing the  $\beta$ -lactam ring to produce penicilloic acid. It can be used to prevent the antibacterial action of penicillin in culture media when preparations of this antibiotic are being tested for sterility.

(See the pharmacopoeial sterility tests for benzyl-, benzathine- and procaine penicillins.)

#### PRODUCTION OF PENICILLINASE

Bacillus species give the highest yields but in many cases, e.g. *Bacillus subtilis*, the enzyme is adaptive, i.e. is not produced unless its substrate, penicillin, is in the environment. Therefore, to obtain high yields, penicillin must be added to the medium in which the organism is grown.

Recently, Pollock (1957) reported on the use of a mutant strain of *Bacillus cereus*. For this organism, penicillin is a constitutive enzyme, i.e. is a constant member of its enzyme complement, and consequently, penicillin need not be added during production. B.P. penicillinase is obtained from this strain.

The organism is grown in conical flasks in shallow layers of a special casein hydrolysate medium. The latter gives very high yields, it contains citrate to remove calcium ions which are essential for the formation and action of a penicillinase-destroying proteinase.

The culture is left at any convenient temperature between 18° and 37°C until growth is apparent and then it is shaken continuously, at 35° to 37°C for

16 hr, because efficient aeration is necessary for maximum penicillinase production

The cells are separated by centrifugation and the supernatant sterilised by membrane filtration (q.v.). Sintered glass and asbestos filters should not be used because they adsorb most of the enzyme. The solution must be kept at 0° to 2°C and used within 2 to 3 days, if freeze-dried and stored in hermetically-sealed ampoules full activity is retained for several months.

One ml of the filtrate will destroy at least 500 mg of penicillin per hour at 30°C and pH 7.0. However, when the penicillin concentration in the reaction mixture is low, i.e. 600 mcg/ml or less, the enzyme is no longer saturated with substrate and cannot work at full efficiency, consequently, the hydrolysis rate falls appreciably. To overcome this and ensure completion of the reaction in the normal time, more enzyme is added. Pollock suggests ten times the amount theoretically necessary under conditions of saturation.

#### OTHER IMPORTANT FACTORS IN THE STERILITY TESTING OF PENICILLIN PREPARATIONS

##### *1 Preservatives in the Penicillinase*

Commercial penicillinase sometimes contains a preservative which must be inactivated appropriately. For example, in one case 0.25 per cent of phenol is used and therefore, the amount of broth in the test should be sufficient to dilute the required volume of penicillinase about 50 times.

##### *2 Bactericides in the Penicillin Preparation*

Multi dose injections and creams of penicillin contain a bactericide, one function of which is to prevent the multiplication of penicillinase producers accidentally introduced during use. This must be suitably inactivated.

##### *3 Procedure for Inactivating the Antibiotic*

Inactivation may be carried out either

###### *(a) Before the Sample is Added to the Broth* This is the method recommended in the B.P.

Sufficient preparation to provide adequate samples for the test and control containers is mixed with the calculated amount of penicillinase and incubated at 30°C for 1 hr. The fact that the preparation has been diluted with the enzyme solution must be taken into account when the samples are subsequently transferred to the medium, proportionately larger volumes of the mixture are used in order to obtain the prescribed sample of the preparation.

###### *(b) After the Sample has been Added to the Broth*

The required amount of penicillinase is added to each tube. The first hour of incubation must be at 30°C even if a different temperature is used subsequently. The organisms must not be added to the control tubes until after the inactivation period because they might be destroyed by the antibiotic. By using this method it is easier to comply with the pharmacopoeial requirement that the inactivation must take place 'under conditions which will prevent the production of undue acidity'. Because the penicillin is divided between several tubes, the amount of acid produced in each is small and is well diluted by the large volume of broth (normally 50 ml) that is used. The buffering capacity of the broth further minimises pH change.

#### *4 Type of Organism used in the Controls*

It must be penicillin sensitive (p. 463).

#### C SEPARATION FROM THE INHIBITOR

Most of the antibiotics discovered since penicillin are also active in very high dilutions but, in addition, it has been difficult to find satisfactory inactivators. The resulting problems are well illustrated by the attempts to develop a reliable sterility test for Streptomycin.

The 1951 Addendum to the B.P. 1948 used the aldehyde reagent hydroxylamine hydrochloride as an inactivator because it combines with a —CHO group in the antibiotic. Hydroxylamine has antibacterial activity itself and, therefore, the neutralisation was carried out before the addition of the samples to the test media but, in spite of this, sufficient inactivator was transferred (it is bacteriostatic at 0.001 per cent) to cause inhibition of contaminants in the tubes and the method was abandoned.

The 1960 Addendum to the 1958 B.P. introduced a combination of neutralisation and dilution. Certain sulphhydryl compounds such as thioglycollate and, particularly, cysteine inactivate streptomycin, possibly, since they are reducing substances, by interfering with the action of the antibiotic on an oxidative enzyme system (Williamson, 1957). 0.2 per cent of cysteine hydrochloride was included in the medium which was adjusted to a post sterilisation pH of 6.5 to 6.6, because acidity reduces the activity of streptomycin which is most effective at slight alkalinity. The recommended volume of medium was sufficient to reduce the final concentration of streptomycin to not more than 400 units/ml.

The neutralising power of cysteine is insufficient completely to inactivate this level of antibiotic but assistance was given by other sulphhydryl compounds.

in the medium, particularly when thioglycollate or cooked meat was used. It was assumed that the residual concentration of the antibiotic was not sufficient to inhibit the majority of organisms.

This method had two disadvantages—

- 1 It would not detect contaminants sensitive to the residual streptomycin concentration
- 2 It involved considerable dilution of the sample with consequent loss of sensitivity, e.g.—

When no dose was prescribed for Streptomycin Sulphate Injection the B.P. recommended 330 mg in 1 ml.

If the injection is packed in a multi-dose container, the sample size in the sterility test is 1 ml.

Since Streptomycin Sulphate B.P. contains not less than 700 units/mg 1 ml of injection of the recommended strength was equivalent to not less than  $330 \times 700 = 231,000$  units.

Therefore, to give a concentration in the medium of 400 units/ml this had to be diluted

$$\frac{231,000}{400} = 578 \text{ times}$$

i.e. with over half a litre of medium.

The situation was even worse with other official antibiotics (bacitracin, oxytetracycline hydrochloride, polymyxin B sulphate and tetracycline hydrochloride) because no suitable antagonists were available. The

1948 B.P. relied upon very high dilution. Two levels were used but the possibility of residual antibacterial activity, particularly at the higher level, and the need to use tiny samples (the alternative being enormous volumes of medium) made the method far from satisfactory. The antibiotic was aseptically dissolved in Water for Injection and 1-ml and 0.1-ml amounts were added to 15-ml tubes of thioglycollate medium. The concentration of the aqueous solution was specified. As an example, it was 5,000 units/ml for Tetracycline Injection, this meant that since tetracycline hydrochloride contains not less than 950 units/mg the 1 ml and 0.1 ml samples contained only about 5 and 0.5 mg respectively. These are very small amounts compared with the doses of 100 to 500 mg normally included in containers of this injection.

However, there is another method by which inhibitory action in sterility tests can sometimes be overcome. Contaminating organisms can be separated from the preparation under test and, after washing, transferred, free from inhibitor, to the culture media. This is the basis of the filtration technique recommended in the 1963 *British Pharmacopoeia* for antibiotics, other than benzathine, procaine, and benzyl-penicillin, and discussed later in this chapter.

(For further information on the neutralisation of inhibitory effects in sterility testing see Klarmann, 1950, Michaels, 1950, and Sykes, 1956.)

### Tests on Aqueous Solutions

The performance of sterility tests on aqueous solutions involves simple aseptic transferences only, and reference should be made to chapter 24 where tests on single dose injections of small volume, multi-dose injections and single dose injections of large volume were used to illustrate three types of transfer. The following additional points are important.

#### I RECORDING THE TEST

A large number of factors must be taken into account in the design of a sterility test e.g.—

The labelling of the containers of medium.

The sample volume or weight

The type or types of medium

The volume of medium

Neutralisation of inhibitory substances

The types of control required

Suitable species and dilutions of organisms for the positive controls

The incubation conditions

These are more likely to receive full consideration if a record form is devised and carefully filled in before the test is started. An example is given in Table 25.1. It is divided into three sections because the whole table is not always required.

Section A is always essential. Tests are useless unless controls are performed to show that the amount of added medicament does not inhibit small numbers of organisms.

Section B is necessary, to confirm sterility and proper formulation and preparation, when new batches of media are brought into use.

Section C is required when substances, such as penicillinasce, additional to the material under test are added, to confirm their sterility and freedom from inhibitory effects.

To illustrate fully the use of the table a relatively complicated example has been chosen and the assumption has been made that new batches of media are being used. Often only Section A is necessary.

## II CHOOSING THE METHOD

Influencing factors are—

### A. The Type of Injection

If this is—

#### 1 SINGLE DOSE AND OF SMALL VOLUME

Decide—

##### (a) If the Medicament is Inhibitory

- (i) If it is, use the appropriate method of neutralisation
- (ii) If it is unlikely to be, e.g. Aneurine Hydrochloride and Cyanocobalamin Injections (these substances are growth factors for certain microorganisms), carry out the test in separate aerobic and anaerobic media, or a joint medium and set up the main positive control (see Table 25 I) as a safeguard

##### (b) If the Injection was Sterilised by Heating with a Bactericide

If it was, neutralise the bactericide appropriately—

Chlorocresol—by dilution

Phenylmercuric nitrate—by using thioglycollate medium

##### (c) For Injections Sterilised by Filtration

if a Bactericide has been Included

If so, use a suitable method of neutralisation—

Phenol, cresol, chlorocresol, hydroxybenzoates—by dilution

Chlorobutol, benzyl alcohol—by dilution

Phenylmercuric borate nitrate or acetate—with thioglycollate medium

#### 2 MULTIPLE DOSE

Decide—

##### (a) If the Medicament is Inhibitory

If it is neutralise appropriately

##### (b) As 1 (b)

##### (c) If a Bactericide has been Included

Only exceptionally will this not be the case (see chapter 20), therefore, neutralise appropriately

#### 3 SINGLE DOSE AND OF LARGE VOLUME

As the medicament will not be inhibitory and the preparation will not contain a bactericide, proceed as in 1 (a) (ii)

### B The Strength of the Injection

1 If the solution is very hypertonic (e.g. Dextrose 50 per cent, Nikethamide 25 per cent, Sulphadimidine Sodium 33 per cent) it is important not to use very small volumes of media because the resulting high osmotic pressure might inhibit the multiplication of contaminants. Although 15 ml of medium is not made harmfully hypertonic by the addition of 1 ml samples of any of the examples quoted, adverse effects might result if the same samples were added to 5 ml of broth

2 If the injection is a strong solution of an antibacterial substance the amount of neutralising agent in the medium may not be sufficient

For example, in the section on the inactivation of the sulphonamides it was stated that 250 ml of an 0.01 per cent solution of PABA in broth (i.e. 25 mg PABA) will neutralise up to 5 G of sulphamamide. Assuming the same equivalence for sulphadimidine sodium 5 G will be neutralised by 25 mg PABA. A 1-ml sample of a 33 per cent injection of sulphadimidine sodium will contain  $\frac{1}{3}$  G of the medicament, and to neutralise this

$$\frac{25}{5 \times 3} = 1\frac{2}{3} \text{ mg is required}$$

Therefore if the broth contains 10 mg/100 ml approximately 17 ml is necessary, i.e. 25 (not 15) ml should be used

### C Precipitation due to Reaction between the Medicament and Broth Constituents

Medicaments that cause difficulty in this respect include concentrated solutions of calcium salts (e.g. Calcium Gluconate Injection) and certain soaps (e.g. Ethanolamine Oleate Injection) and cationic detergents. The resulting turbidity makes the test results difficult to read, and subculturing after the normal incubation period may be necessary to decide if bacterial growth has taken place. The usual method of doing this is to transfer 1-ml amounts into fairly large volumes of broth (e.g. 50 ml) so that the turbidity from the sample is no longer very noticeable and to continue incubation for a further 2 days. This is sufficient time for a subculture because if the subcultured sample contains organisms they will be present in large numbers and, probably, will be actively dividing, therefore, the lag will be short and a detectable turbidity quickly produced.

### III LABELLING THE CONTAINERS

After a series of tests a large number of containers may require incubation and unless each is labelled

Table 25 /  
Sterility Test on Multiple dose Benzypenicillin Injection containing 150 mg (approximately equivalent to 250 000 units) per ml  
and 0.5 per cent phenol as a bactericide

		Label	Medicament	Medium	Type	Neutralisation by	Organisms 1 ml of a 1 in $1 \times 10^8$ dilution of an 18-24 hr culture of	Inhibition	Ex- pected	Ob- served	Result
Test	Aerobic	TA	1.3 ml of neutralised solution, equivalent to 1 ml of injection	50	Nutrient broth	(a) Penicillin—adding 1.5 ml of Penicillinase Solution B P to 5 ml of injection, mixing and keeping at 30°C for 1 hr	<i>Staphylococcus aureus</i>	do	—	—	—
	Anaerobic	TN	do	do	Cooked meat	(b) Phenolic bacteria in the penicillinase—by dilution	<i>Clostridium histolyticum</i> (Penicillin sensitive)	do	—	—	+
A	Main (Positive) Control	CA	do	do	Nutrient broth						—
	Anaerobic	CN	do	do	Cooked meat						+

<b>B</b>	Negative Control	Aerobic - Anaerobic	-A - -N	do	Nutrient broth	do	-	-
	Positive Control	Aerobic - Anaerobic	+A - +N	do	Nutrient broth Cooked meat	do	Staph aureus (Oxford)	do +
<b>C</b>	Negative Control	Aerobic - Anaerobic	-A - - -N -	do	Nutrient broth Penicillinase BP	do	Cl histolyticum	do +
	Positive Control	Aerobic - Anaerobic	+A + - +N +	do	Nutrient broth Cooked meat	do	-	-

neatly and in a way that distinguishes it from all others great confusion can occur when the results are examined

When tubes are used there is insufficient space for detailed information. Codes are useful but they should be simple, of general application and always recorded in the table. The codes used in Table 25 I would not distinguish that test from any other and, unless the containers for each test are kept separate in an appropriately labelled basket or rack, additional coding is necessary on each container (e.g. P. for penicillin, in the example given).

When a joint medium is used, TAN is a suitable coding for the test. The control labels are the same as when different aerobic and anaerobic media are used, because controls must always be separate even if a joint test is performed (see p. 453).

Glass pencil marking must be perfectly legible and not smeared by careless handling. Non self adhesive paper labels should be held on by elastic bands.

#### IV THE SAMPLE SIZE

The following typical examples are largely based on the B.P. sampling directions.

##### *1-ml Ampoule*

Since the volume is less than 2 ml half must be used for a test for aerobes and half for a test for anaerobes. The same volumes must be added to the main controls and therefore, a second ampoule must be used. It is not permissible to use a quarter of one ampoule for each of the tests and controls because then the samples would be only 50 per cent of the pharmacopeial minimum.

##### *2-ml Ampoule*

1 ml is used for each test and control and, again two ampoules are needed.

##### *5 ml Ampoule*

As for 2 ml ampoule except that one ampoule is sufficient.

##### *10-ml Multi dose Injection*

As for 5 ml ampoule

##### *500-ml Infusion Fluid*

1 ml samples would comply with the B.P. requirement but would not adequately represent the contents of the bottle, 10-ml volumes are more appropriate.

the preparation has been diluted by the inactivating solution must not be overlooked. The volumes sampled from the mixture should be increased appropriately, Table 25 I gives an example.

#### THE USE OF JOINT MEDIA

When a joint medium is used the Therapeutic Substances Regulations require double the normal sample size so that the same volume is tested as when separate media are chosen. In some cases this necessitates larger volumes of media to ensure an excess of inactivator or to prevent excessive dilution of the nutrients.

#### V THE VOLUME OF MEDIUM

This is influenced by—

- The need to dilute out the effect of a bactericide or an inhibitory medicament
- The sample size

A useful general rule is 15 ml for a 1-ml sample, 25 ml for a 2-ml, 50 ml for a 5-ml and 100 ml for a 10-ml.

An example in which both factors operate is the use of a joint medium for testing a multi-dose preparation containing 0.5 per cent phenol as a bactericide. Fifty times is not greatly in excess of the dilution required to inactivate phenol and when a joint medium is used and, therefore, double the normal sample is necessary, the volume of medium must also be doubled, to 100 ml.

#### VI PRE-TREATMENT OF ANAEROBIC MEDIA

Anaerobic media absorb air on storage and may require de oxygenation before use.

Cooked meat medium—unless used soon after sterilisation should be heated in boiling water for at least 20 minutes.

Thioglycollate media—see p. 449.

In both cases, screw caps are loosened during heating but closed immediately afterwards. Before use, thorough cooling is essential to prevent damage to organisms in the test and controls, it should not be hastened by shaking in a stream of cold water because this encourages resorption of air.

#### VII TRANSFERENCE OF SAMPLES TO TEST MEDIA

A sample that is being tested for aerobes can be pipetted on to the surface of the medium but the sample for anaerobes must be introduced carefully.

##### THE EFFECT OF PRE NEUTRALISATION

When neutralisation is necessary before the samples are transferred to containers of media the fact that

(see 'Aseptic Transferences') to the bottom of the container without bubbling air into the medium

Containers of aerobic media can be rotated between the palms to distribute the sample but with anaerobic and joint media, mixing can only be assisted gently because of the risk of reoxygenation. Before the addition of organisms to positive controls admixture is particularly important because when preparations containing inhibitory substances are under test local high concentrations might damage the cells. It is advisable to leave anaerobic control tubes for some time between the additions of sample and organisms so that diffusion can assist mixing.

In some cases, e.g. with 0.5-ml samples, it is possible to transfer the samples to two tubes from the same pipette. With practice, both tubes and the container under test can be held at the same time viz.,

3rd finger	—	Tube CA
2nd finger	—	Tube TA
1st finger	—	Container
Thumb	—	

For example, when testing a 1-ml ampoule the complete contents are taken into a capillary pipette, half is transferred to tube TA and the rest to CA. It is slightly easier (because the procedure is exactly the same for both) to inoculate the (aerobic or anaerobic) test and control together than to inoculate both tests or controls.

#### Tests on Aqueous Suspensions

The testing of aqueous suspensions, such as Bismuth Oxychloride, Corticotrophin Zinc Hydroxide, Hydrocortisone Acetate, Propyl iodine and Protamine Zinc Insulin presents the following special problems:

##### 1 OBTAINING A REPRESENTATIVE SAMPLE

The container should be well shaken before opening and, since some sedimentation may occur during the

#### VIII INOCULATION OF CONTROLS WITH ORGANISMS

The choice of organisms for non inhibitory injections is not critical. Frequently, 1 ml volumes of high dilutions (e.g. 1 in  $1 \times 10^3$ ) of 18 to 24 hr cultures of *Escherichia coli* or *Staphylococcus aureus* (aerobic) and *Clostridium sporogenes* or *Clostridium histolyticum* (anaerobic) are used. (A wider range of organisms is essential when new media are being evaluated—see chapter 19.)

If the preparation contains an inhibitor the organisms must be sensitive to it so that they will fail to grow if neutralisation has been unsuccessful. Often the organisms mentioned above are suitable but occasionally, e.g. for certain antibiotics and sulphonamides, they are too resistant. Generally, Gram-positive organisms are preferable because the majority of antibacterial substances are active against them. As an example, *Escherichia coli* is unsuitable for the penicillin test and *Staphylococcus aureus* is used instead, but a special strain (the Oxford H) is necessary because many strains are penicillin-resistant. The names of suitable highly sensitive organisms can often be found in the descriptions of antibacterial agents given in Volume I of the *Extra Pharmacopoeia*.

In a bacteriology laboratory approximately 1-ml volumes of the diluted suspensions are transferred with a Pasteur pipette. Precautions are taken to prevent drops falling from the tip (see chapter 24). After use, the pipette is put into a cylinder of lysol, the air being expelled just before the tip enters the disinfectant to ensure that the latter is sucked into the bore. The teat is left on for several minutes.

latter, the solids should be resuspended before transfer by drawing the preparation into and ejecting it from the pipette several times.

#### 2 READING THE RESULTS

The turbidity produced by the suspension may obscure growth and necessitate subculturing.

#### Tests on Powders

##### A. SOLUBLE POWDERS

Most of the medicaments of the injections that are prepared aseptically shortly or immediately before use are examples (see Appendix 4).

##### 1 POWDER IN SMALL AMPOULE (i.e. 2 ml or less)

Open the ampoule and hold it, together with the containers of media (one aerobic and one anaerobic), ready for transfer.

Take up some of the aerobic medium into a pipette and transfer it to the ampoule. Withdraw and return it several times until solution is complete or almost so. The anaerobic medium should not be used for this purpose because—

- (i) It will become oxygenated
- (ii) The air space in the bottle will be increased, since only half of the liquid used as a solvent is returned
- (iii) For the same reason as (ii), the proportion of (reduced) medium to (oxygenated) sample will be lowered

Transfer to each of the containers a volume corresponding approximately to the recommended sample weights for powders, i.e. 50 mg if the ampoule contains 100 mg or more, and 50 per cent if it contains less than 100 mg. This volume can be estimated without difficulty, particularly if the pipette has one graduation mark (see chapter 24).

## 2 POWDER IN LARGE AMPOULE OR VIAL

In these cases the above procedure is cumbersome because of the large volume of broth needed. It is more convenient to prepare the injection first using the appropriate solvent (usually Water for Injection or Water for Injection free from Carbon Dioxide) and strict aseptic technique. Then the solution is sampled by the method for aqueous solutions. A separate test on the solvent is desirable to confirm its sterility.

## 3 BULK POWDERS (e.g. sodium chloride or citrate in a flat screw-capped jar)

Proceed as follows.

Use 1-oz McCartney bottles of media, the aerobic and the anaerobic almost full. Loosen their caps after labelling them appropriately.

Place the jar to the right of the bottles of media after swabbing and loosening its cap.

Flame a nickel spoon, allow it to cool, raise the lid of the jar and remove about 50 mg of the powder. (Some time before attempting this type of exercise a few weighings of different powders should be made to facilitate the estimation of 50 mg by eye.) Replace the jar lid.

Lift the cap of the first bottle but keep it above the mouth of the bottle for protection. Pour in the powder from the spoon and replace the cap at once.

A sample significantly larger than 50 mg should not be used because it might make the medium excessively hypertonic.

## 4 OTHER PROBLEMS

These include

- (a) *Inhibitory medicaments, e.g.*  
Tryparsamide—use thioglycollate medium containing cooked meat  
Penicillins—use penicillinase  
Other antibiotics—use the filtration method
- (b) *Production of an unfavourable pH, e.g.*  
Barbiturates—use dilution
- (c) *Precipitation, e.g.*  
Barbiturates—subculturing may be necessary

## B INSOLUBLE POWDERS (e.g. talc and other ingredients of dusting powders)

After the powder has been transferred to the media by the method described for bulk soluble powders the aerobic containers are tightly closed and shaken vigorously to encourage organisms to separate from the particles. This is repeated at intervals during the first three days of incubation. Except during shaking the caps are left slightly unscrewed to prevent oxygen lack inside. For the last two or more days the containers are not disturbed. The powder may settle leaving a clear supernatant that indicates sterility but subcultures will be necessary if turbidity remains.

The anaerobic containers should not be shaken because this causes oxygenation. Instead, the powder is tapped gently down to the region of low oxidation reduction potential at the bottom of the bottle. It is easier to achieve this in media without agar, e.g. cooked-meat medium or thioglycollate broth U.S.P.

If the powder is so light that it will not sediment sloppy agar can be used as the anaerobic medium. The sample is put into an empty jar or tube and melted but cool and almost-setting sloppy agar is poured over it. The powder is prevented from rising by the high viscosity of the medium.

Inhibition is not often a problem with insoluble powders but some samples of zinc oxide are antibacterial probably due to traces of soluble zinc compounds. Dilution in a large volume of broth will usually overcome this effect.

## TESTS ON WATER-MISCIBLE SEMI-SOLID PREPARATIONS (e.g. creams and mucilages)

These are treated as insoluble powders but it is more convenient to use

- I A spatula, instead of a spoon, because detachment of the sample is easier

2 Wide-mouthed jars of culture media, to facilitate introduction of the preparation

The anaerobic sample should be carried to the bottom of the container. Catheter lubricant (Compound Paste of Tragacanth B.P.C. 1954) has a base

of tragacanth mucilage and contains phenylmercuric nitrate. The latter must be neutralised by thioglycollate medium but, although this is a joint medium, the aerobic and anaerobic tests should be performed separately to permit shaking of the former.

### Tests on Oils

Oils and semi solid anhydrous materials present some of the most troublesome problems in sterility testing.

- 1 They are difficult to disperse in aqueous test media. Usually oils form a surface layer and ointments float in lumps.
- 2 Adequate dispersion makes the media turbid and necessitates subcultures.
- 3 Contaminants are coated with an anhydrous film and unless this is broken to bring the organisms into contact with the nutritive aqueous medium multiplication is unlikely to occur.
- 4 A thick layer of oil on an aerobic medium may lead to semi anaerobic conditions below and reduced recovery of organisms.
- 5 The efficiencies of new methods are difficult to evaluate.

### METHODS

#### A Vigorous Shaking

##### 1 OILS

Shaking is the basis of the *British Pharmacopœia* technique for oily solutions and suspensions.

The media should be in screw capped bottles or jars.

The samples are added and the containers tightly closed and vigorously shaken. Ideally, shaking should be continued throughout the incubation period but this is not always possible because it requires a shaker in the incubator, therefore, a thorough shake by hand at least once a day is sometimes used instead.

A wide jar is better than a narrow bottle because the oil when it separates produces only a thin and incomplete film over the larger surface. Bullock and Booth (1953) suggest a jar of 6.5 cm internal diameter for a sample of 1 ml.

After incubation subcultures are made if necessary. Occasionally, particularly with aerobic media, the oil separates quickly and the absence or presence of growth in the broth below is clearly distinguishable.

Although this is a satisfactory test for aerobes it is not ideal for anaerobes. Shaking oxygenates the medium and prevents the growth of exacting species. Without shaking the preparation remains at the top of the liquid where the oxidation reduction potential

### and Ointments

is far from ideal. However, several precautions can be taken to make the method more satisfactory for the recovery of this type of organism.

- (a) Thioglycollate medium should be used because its reducing agents can partially restore reduced conditions after shaking. It must contain agar, because agar free broth oxygenates too readily, and be quite fresh, i.e. not reheated.
- (b) The containers should be almost full, then there will be very little air inside when the sample has been added.
- (c) Shaking should be minimal, not more than once a day.
- (d) Subculturing should be carried out even if it seems unnecessary. Organisms that have been separated from the oil by shaking but have not found satisfactory conditions for multiplication may grow when transferred to the unshaken subculture medium.

Alternatively, the anaerobic test can be performed in the following way, which is based on a method used by Coulthard (1935).

A volume of oil in excess of the official sample is added to a bottle or jar of molten nutrient agar cooled to about 45°C., at which temperature contaminants will not be harmed. After tight replacement of the cap the bottle is gently inverted and reverted until the medium solidifies. The aim is to break the oil into small globules (averaging about 2 mm) but they must not be so tiny that they make the agar turbid. The medium must be warm enough to allow distribution of part of the sample to the bottom of the bottle before gelling begins. Contamination is shown by growth around the globules and splits, due to gas production, in the agar. The large sample size compensates for the fact that only contaminants at the surface of the globules will be detected.

*Note* When sampling oils a special Pasteur pipette with a wide capillary is necessary.

#### 2 SEMI SOLID OILY MATERIALS (e.g. eye and other ointments)

Semi solids cannot be dispersed satisfactorily in a cold aqueous medium. One partial solution is to

use very large samples in the hope that contaminants on the surfaces exposed to the medium are numerous enough to reveal infection

#### *Aerobic Test*

Use 4-oz wide-mouthed screw-capped jars containing 50 ml of nutrient broth

##### (a) Tube of eye ointment

Remove the cap of the tube

Remove the previously loosened lid of the broth jar but hold it just above the mouth as a protection against aerial bacteria

Squeeze several inches of ointment on to the surface of the medium Replace the lid and shake

##### (b) Jar of ointment

Remove the lid of the jar but use it for protection, as above

With a flamed but still very slightly warm nickel spoon lift out a large piece of ointment from the centre of the contents

Transfer the sample to the broth If the spoon is warm, little difficulty is experienced but if it is cold a second spoon, or a spatula, may be needed to detach the ointment

Replace the lid and shake

(Before the spoon is flamed for re use it should be wiped with a cotton-wool swab to prevent the spattering of hot 'fat' over the working area)

#### *Anaerobic Test*

Transfer the sample to the bottom of an empty sterile 4-oz wide-mouthed screw-capped jar and pour over it 50 ml of sloppy agar at 45°C Flame the mouth of the tube before pouring, hold the jar near to horizontal and pour gently to avoid entrapped air bubbles

The following is a better method

Warm about 4 ml of light liquid paraffin to 50°C in a thermostatically controlled water bath The paraffin is previously sterilised by dry heat in a shallow screw-capped jar

Then add, aseptically, about 200 mg of the sample This will melt quickly unless it contains beeswax, when the paraffin must be at about 60°C If necessary, return to the bath for the minimum time needed to ensure mixing

Finally, without delay and using a warm (from flaming), but not hot, wide bore capillary pipette, transfer slightly more than 1 ml amounts to nutrient broth (aerobic test) and nutrient agar at 45°C (anaerobic test) Continue as for an oil

A disadvantage of this technique is the short exposure of the sample to 50° or 60°C which may harm very sensitive vegetative bacteria Also, a separate test is necessary to confirm the sterility of the liquid paraffin

#### *B Emulsification*

An emulsifying agent is used to produce satisfactory dispersion

One of the first substances tried was saponin but this was too toxic It has a harmful effect on cell membranes, as is illustrated by its haemolytic action on red blood cells

More recently, Rygiel (1961) has reported on the use of a non ionic surface active agent Unlike many cationic and anionic surfactants, the non ionic types are rarely harmful to micro-organisms and sometimes enhance their growth. The substance chosen by Rygiel (Tyloxapol—an oxyethylated tertiary octyl phenol polymethylene polymer) was shown to have no deleterious effects on bacteria

It was included at a concentration of 2.5 per cent in Fluid Thioglycollate Medium U S P and it did not affect the pH and clarity of this medium.

120 ml of the medium was sterilised in a 500-ml conical flask, and cooled About 10 ml of the sample was added aseptically and emulsified by magnetic stirring (This is an excellent method of stirring when asepsis must be maintained, a sterile, P T F E-covered, magnetised bar is operated inside the flask by a rotating magnet beneath the base)

After 3 minutes of stirring the flask was incubated for 7 days, and 1-ml subcultures were taken into 40-ml volumes of normal Fluid Thioglycollate Medium on the second and fifth days Although turbidity obscured growth in the initial container the contents became yellow when contaminated.

Oxygenation caused by the stirring will reduce the suitability of the original medium for anaerobes but any present in the subcultured volumes will grow in the fully-reduced subculture medium.

#### *C. Filtration*

See Filtration Methods of Sterility Testing

#### *D Centrifugation*

The sample is dissolved in sterile light petroleum (B pt 40°–60°C) and centrifuged The supernatant liquid is separated from the deposited organisms, more solvent is added, centrifugation is repeated and the supernatant again discarded

The last traces of solvent are removed from the deposit under reduced pressure, broth is added and the tube incubated

For further information, see Bullock and Booth (1953)

### Controls

The usual controls are necessary. Traces of certain unsaturated fatty acids present in natural oils and fats are toxic to some organisms and, therefore, the main control is necessary even when the oil or base contains no medicament.

### EVALUATION OF NEW TECHNIQUES

To prove a new method it is necessary to show that it can transfer the contaminants of oily materials from their anhydrous environment to aqueous culture media and encourage them to multiply. Therefore, research must be done with oily preparations that have been contaminated previously. Organisms from a broth or aqueous suspension or even from a slope or plate of solid medium are unsuitable for this purpose because they are enclosed in droplets or films of moisture and will be recovered from the oily

media more easily than the dry organisms that constitute the majority of normal contaminants.

The most convenient source of dry organisms is soil that has been shown by previous experiments to contain aerobes and anaerobes capable of withstanding drying at warm room temperature. After admixture with soil dried in this way the oily material is tested by the new method.

The use of specific exacting aerobic and anaerobic bacteria facilitates reproduction of experiments but there are many difficulties associated with the preparation of suitable dry organisms (see, for example, Bullock and Keepe, 1951).

### INHIBITORY MEDICAMENTS

These must be taken into account. For example, PABA-containing media are used for sulphacetamide eye ointment and penicillinase treatment is necessary for penicillin ointments. In the latter case emulsification is desirable to ensure contact between the enzyme and all of the antibiotic.

### Tests on Ligatures and Sutures

Catgut and kangaroo tendon, the absorbable materials used for ligatures and sutures, may be sterilised by three methods:

- 1 Heating in an anhydrous fluid
- 2 Cold treatment with iodine
- 3 Exposure to ionising radiations

The normal methods of packaging are—

- 1 Heat-treated—in fused glass tubes or in plastic envelopes within a glassine paper outer
- 2 Iodine-treated—in fused glass tubes
- 3 Radiation-treated—in envelopes of aluminium foil or plastic aluminium foil laminate

The containers are filled with a tubing fluid that keeps the gut flexible without causing undue swelling. Common solutions are 96-97 per cent of ethyl alcohol and 90 per cent of isopropyl alcohol, to these a bactericide, which is often a mercurial compound (e.g. phenylmercuric acetate), is usually added.

Several problems complicate the testing of these materials.

### I DEEPLY EMBEDDED CONTAMINATION

Catgut is made from the intestines of sheep (see Cooper and Gunn, 1957a) which in the slaughter-

houses often become heavily contaminated with the spores of dangerous pathogens such as *Clostridium tetani* and the *Clostridium* spp responsible for gas gangrene. During manufacture these may be trapped deep inside the threads, and as it is essential to detect any that escape sterilisation the incubation period of the sterility tests is lengthened to 12 days (*British Pharmacopœia*, 1963 and *Therapeutic Substances Regulations*, 1963). This gives time for the culture medium to permeate the threads and the organisms to recover and multiply into a detectable turbidity. As additional precautions, the whole of the contents of each container must be used, and repeat tests are not allowed.

### 2 PRESERVATIVES IN TUBING FLUIDS

These must be inactivated. The alcohols present no difficulty because the amounts transferred to the media in or on the drained gut are insufficient to inhibit bacterial growth. However, the nature of the bactericide must be ascertained and appropriate steps taken.

Traces of iodine and its compounds may remain in iodine sterilised gut; these require neutralisation.

At one time heat sterilised gut was sometimes packed in an anhydrous tubing fluid and called 'Boilable catgut'; this fluid had to be soaked out thoroughly before the gut was transferred to a culture medium.

Suitable treatments are—

(a) *For Iodine-sterilised Gut*

- 24 hr incubation at 31°C in 50 ml of sterile water to soak out the tubing fluid, followed by
- 24 hr incubation at 31°C in 50 ml of a sterile solution containing 1 per cent sodium thio sulphate and 1 per cent sodium carbonate, to neutralise residual iodine, and
- 12 days incubation at 31°C in 50 ml of a culture medium containing, if necessary, a suitable inactivator for the bactericide

(b) *Heat and Radiation sterilised Gut*

As for iodine sterilised gut but excluding stage (ii)  
Stage (i) is particularly important for boilable gut

### 3 MANIPULATION

Great care is required to avoid accidental contamination during the transferences Useful precautions are—

(a) Make no attempt to divide the coil because the elasticity of the gut often results in the cut pieces falling to the bench and uncoiled strands fouling the mouths of containers Preferably choose a joint medium but if separate media are preferred use an entire tube of gut for each

(b) Use wide mouthed jars, rather than large tubes for the treatment fluids and media, this lessens the risk of touching the neck Also, in tubes, the coil sometimes adheres to the glass above the medium, and additional manipulations, often complicated by uncoiling, are involved in pushing it below the surface

(c) Use long (e.g. 8 in.) forceps

### TESTING PROCEDURE

1 Sterilise the Outside of the Container

(a) *Tubes* Immerse in a beaker of 10 per cent lysol for 10 minutes (The U.S.P. recommends soaking in methylrosaniline chloride (crystal violet), or other suitable solution, for 18 to 24 hr, use of a dye tests the seals and ensures that only sound tubes are subjected to the sterility tests)

(b) *Paper Envelopes* Hold in forceps and paint with a swab soaked in Weak Iodine Solution B.P. Allow to dry

(c) *Foil Packs* Treat overnight in a solution that is sometimes recommended for the storage of these containers—97 per cent isopropyl alcohol containing 1 per cent of formaldehyde

After methods (a) and (c) the container can be dipped into a beaker of sterile water to reduce the risk of transferring traces of adherent disinfectant

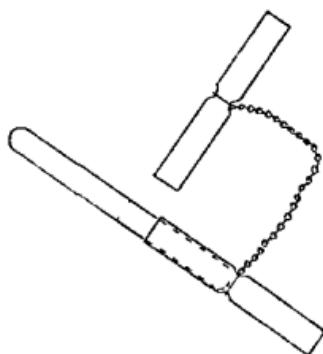


Fig. 25.2 CATGUT TUBE BREAKER

to the culture tubes Then the surface is well-drained or blotted between folds of sterile cloth

2 Open the Containers

*Tubes* These are best opened with a sterile catgut-tube breaker which consists of two stainless steel tubes that fit over the ends of the gut tube (Fig. 25.2) and through which pressure can be applied to crack the tube at the file mark around the centre If this mark has not been put on by the manufacturer it should be made before the tube is disinfected.

*Envelopes* These are cut open, near one end, with sterile scissors, any outer envelope is discarded first

- Remove the coil with sterile forceps Hold as much of the gut as possible to prevent the ends from breaking loose Forceps, for this purpose, should be sterilised by autoclaving or dry heat and not by flaming, because several inches are introduced into sterile containers
- Lift the previously-loosened lid of the container of sterile water and drop the coil inside Incubate
- If appropriate, transfer to the iodine neutralising fluid and reincubate
- Transfer to thioglycollate or other appropriate inactivating medium If inactivation is not necessary use one of the following—
  - Thioglycollate—as a joint medium
  - Sloppy agar—as a joint medium (Put the coil into a sterile jar and pour molten sloppy agar, at about 45°C, over it.)
  - Separate aerobic and anaerobic media, with separate tubes of gut
- Incubate for 12 days at 31°C

## OTHER LIGATURES AND SUTURES

Most of the non absorbable materials, i.e. horsehair, linen, silk, silkworm gut, nylon and metals, can be sterilised by one of the normal heating methods—

usually autoclaving. When they are supplied in a tubing fluid this is often water, with or without a bactericide. Direct transference from the container to the culture medium is usually possible.

### Tests on Surgical Dressings

#### PROBLEMS

1 Cotton wool, gauze, lint, bandages and adhesive plasters are examples of dressings that may be required sterile. Before sterilisation, cotton wool may be heavily contaminated because its preparation involves a drying process in which hot air is blown through it, causing retention of air borne bacteria by the fibres. Although fabrics, such as gauze, are more lightly contaminated the bacterial load on dressings in general is more than on most other pharmaceutical and medical items. As an example, gauze may carry about 100 organisms/in<sup>2</sup>. Since dressings are used in direct or close contact with wounds or, in the case of gauze, within the operation field during surgery the method of confirming sterility must be reliable.

2 With the exception of some sizes of plaster and bandage, dressings are fairly large. This makes aseptic handling difficult, particularly as it is necessary to unroll or unfold the dressing to take a sample from the centre where sterilisation failure is most likely. This is not a problem with small items because they can be inoculated whole.

3 When the outer wrapper of a sterile dressing is opened organisms on its surface are scattered into the air of the screen. These, assisted by the air movements caused by the complicated handling of the dressing, may contaminate the sample. This problem is accentuated by the large area of material exposed and the long exposure time.

It follows that, even with a skilled worker and good aseptic precautions, a positive result may be due to contamination during manipulation. Savage (1940) using 69 dressings known to be sterile obtained 16 positives and from this and other experiments concluded that it would not be unusual for up to 25 per cent of tests to become infected adventitiously. However, more recently, Tattersall (1961) has claimed that, without the use of a sterile room or screen, contamination can be reduced to 2 to 3 per cent for difficult dressings and about 1 per cent for individually wrapped adhesive dressings. To allow for this important source of error it is necessary

1 To include with the dressings under test a series of known sterile dressings as a check on the operator's technique and the bacteriological condition of the atmosphere.

- 2 To make some allowance for adventitious contamination of the test series to minimise the probability of wrong rejection of a sterile batch.

The test for dressings in the *British Pharmaceutical Codex*, which is based on a method devised by Savage (1940), takes these points into account—

- 1 Ten dressings (or portions of these) and 10 control dressings, similar to those under examination except that they are certainly sterile as a result of careful laboratory sterilisation, are tested in random order at the same time and place. Each is inoculated into a separate container of aerobic medium.
- 2 The sample is considered sterile in respect of aerobic organisms if none of the control series and not more than 3 of the test series show growth.

The whole procedure is repeated for anaerobes and interpreted in the same way.

The statistical basis of this test has been fully discussed by Bryce (1956) and 'for the non mathematical' he justifies the interpretation in the following way:

'If no positive appears in the control series we may infer that contamination of a tube by chance is a fairly unlikely event. That being so, two such events will not frequently occur together, three most infrequently and four is so unlikely that we infer that chance is not a sufficient explanation.'

Savage has stated that, since dressings are heavily contaminated before sterilisation, failure of the process will always result in overall lack of sterility in the batch and, therefore, 10 positives in the test series. If correct, this further justifies the assumption that seven negatives indicate sterility, particularly if a skilled worker can adventitiously contaminate up to 25 per cent of his transfers. As Savage (1961) more recently commented, this assumption of overall lack of sterility may not always be justified, e.g. improper loading of a large steriliser might give both sterile and unsterile products, in these circumstances the test becomes less precise.

When the number of positives is at or near the limit, i.e. 3 or 4, retesting is allowed because the result is most likely to be inaccurate at these points (Bryce, 1956). The repeat is performed with samples of larger size not, as is sometimes the case for other

products, with a larger number of samples. This has the advantage of increasing the probability of discovering lack of sterility without affecting the adventitious contamination rate.

#### TECHNIQUE OF THE TEST

Although this is based on Savage's method (1940), with which he was able to reduce the rate of accidental contamination to 8 per cent several modifications are included.

- 1 Perform the test in an asepsis room provided with bacteriologically clean air.

- 2 Decide on a convenient line along which to cut the external wrapper. (Most sterile dressings are doubly wrapped.) About  $\frac{1}{2}$  m from one end is suitable. Paint this region with Weak Solution of Iodine B.P. and leave the packet resting on the teat end of the pipette rack (i.e. with the painted area out of contact with any surface) for 5 min. This allows time for the disinfectant to act and the solvent to evaporate. Then cut along the line with a sterile scalpel or pair of sharp scissors. Alternatively, the cut can be made with a very hot iron, e.g. thick copper wire beaten into a blade at one end and mounted in a handle at the other.

- 3 Hold the packet in the L.H. and gently draw off the smaller part of the wrapper with forceps and drop it into the waste container.

- 4 Hold the exposed inner wrapper with forceps and pull off and discard the rest of the outer.

- 5 Open the inner wrapper in the same way. Scorching is preferable unless it is certain that the iodine solution will not penetrate the paper (many glassine papers can be safely treated). Disinfection or scorching is unnecessary if the dressing was doubly wrapped before sterilisation or the outer wrapper was separately sterilised and put on aseptically and, in both cases the outer wrapper is impermeable to bacteria (Hunter, Harbord and Riddett 1961). Treatment is necessary in other cases and where the method is in doubt.

- 6 Hold the packet in the L.H., remove the smaller part of the wrapper with forceps and place it gently on the bench.

- 7 Hold the dressing in a fresh pair of forceps and remove the rest of the wrapper to the bench.

- 8 If the dressing is small it can be placed immediately in the appropriate medium. If it is large (e.g. a roll of gauze or lint) hold one end in the fingers instead of forceps during Stage 7. (It is an advantage to wear a sterile rubber glove on the right hand, this can be put on between removing the inner and outer wrappers, the packet being placed

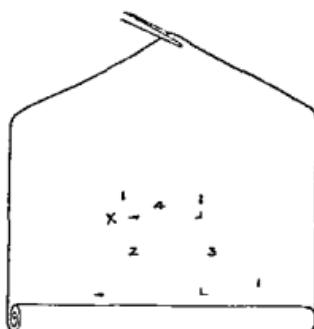


Fig. 25.3 SAMPLING A LARGE DRESSING

Cuts are made along the dotted lines but the sample is left hanging by a few threads at X and then pulled off with forceps.

on the sterile tile meanwhile.) Lift the roll to the top of the screen, grasp the free edge of the fabric with forceps in the L.H. Carefully unroll the rest with the R.H., holding the roll as near to the side as possible, until it reaches the tile below. Then, with sterile scissors cut out a piece at least  $4\text{ cm}^2$  in area from near the centre and close to the bottom (i.e. near the middle of the dressing, leave it hanging by a few threads (Fig. 25.3). Pull off the piece with long forceps. Because there is a lower risk of laboratory contamination if the sample is kept small Savage estimated the smallest size at which the chance of obtaining a sterile sample from contaminated material would be negligible and he arrived at  $4\text{ cm}^2$ . In fact, samples of 2 to 4 times this area can be handled with little increase in the contamination risk. Cutting should be done with very sharp, long bladed but light scissors.

- 9 Hold the sample quite still for 30 seconds. Savage found that a slight delay at this stage reduced the number of infected containers. He suggested that during this period airborne contamination, created by the preceding manipulations subsides and thus decreases the risk of infecting the containers of media when they are opened.

- 10 The sample is carefully inoculated into wide-mouthed jars containing 50 ml of the appropriate medium. Separate aerobic and anaerobic broths or a joint medium (see Ligatures and Sutures) can be used. The number of tests and controls should not be reduced if a joint medium is preferred. Incubation is for 5 days at  $37^\circ\text{C}$ .

Medicated dressings must be inactivated appropriately

### Tests on Paraffin Gauze Dressing

This is a cotton net impregnated with soft paraffin (see chapter 22) It presents the problems of dressings and oily materials Two methods have been reported

#### METHOD 1 (Developed by Savage (1942) and outlined in the B P C (1963))

A suitable container for a small piece of dressing is a 4-oz screw-capped jar with a card liner In it a layer of light liquid paraffin about 1 cm deep is sterilised by dry heat Afterwards, 50 ml of culture medium is added and the whole is sterilised by autoclaving For use, the contents are allowed to cool to about 50°C or, if sterilisation has been carried out on an earlier occasion, warmed to this temperature

The material is aseptically removed from its container If this is—

a *tin*, remove the sealing tape, swab and remove the lid, fold back the upper pieces of ret and draw one out from the centre

a *plastic envelope*, use the whole piece, this is easier to extract if the envelope is opened by diagonal cuts (Fig. 254)

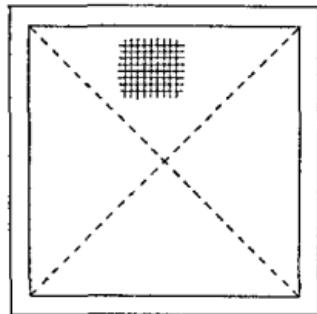


Fig. 254 OPENING A PLASTIC ENVELOPE  
CONTAINING PARAFFIN GAUZE DRESSING  
Cuts are made along the dotted lines

A large continuous length of dressing (see chapter 22) should be inoculated whole into a container of suitable size because it is difficult to remove a piece from its centre

The sample is inoculated into the warm paraffin on the surface of the broth, the base melts and the net falls into the medium below

Twenty tests and twenty controls are performed, as for other dressings Thioglycollate is more suitable than sloppy agar as a joint medium because it can be subcultured more easily

In this method the base is imperfectly tested because the containers are not shaken but, provided the dressing was sterilised complete, sterility of the net indicates a high probability of sterility of the paraffin This conclusion is not justified when the base and dressing are separately sterilised and then aseptically mixed (see chapter 22)

#### METHOD 2 (Developed by Yarlett, Gershenson and McClenahan, 1954, and used in the U S P XVI) The main features are—

1 The medium is Fluid Thioglycollate Medium U S P modified by the addition of 0.1 per cent of agar and 0.5 per cent of gelatin to increase the viscosity and enhance the nutritive qualities The raised viscosity improves the dispersion of the sample and reduces oxygenation during and after shaking

2 The containers are 4 or 16 fl oz wide mouthed jars closed with aluminium or lacquered steel screw caps containing a rubber ring treated with silicone grease to prevent adhesion to the rim of the jar The small size is used for small pieces of dressing (3 in. x 6 in.) and the large one for whole dressings (up to 3 in. x 36 in.)

3 The sample is added to the medium at about 52°C, the lid is fixed tightly with a strong rubber band and the jar is mechanically shaken for 30 sec (small jar) or 10 min (large jar)

4 Afterwards the contents are allowed to cool in a slanting position and the resulting surface layer of congealed paraffin is broken by a single shake to ensure satisfactory aerobic conditions during the subsequent incubation

5 After incubation at 32°C for 14 days the jars are reshaken for the same time as before and 0.5 ml samples are transferred to 15-ml volumes of the medium warmed to 52°C The subcultures are incubated for 4 days because shorter times were found inadequate for the recovery of some anaerobes and species of Nocardia

This technique successfully detected contamination with spores of *Clostridium sporogenes* and a sporing *Bacillus* at levels as low as 4 spores per 3 in. x 36 in sample of dressing

### Tests on Equipment

#### 1. Interiors of Empty Containers

##### (a) *Clinbritic Vaccine Bottle*

Assume this has a paper cap tied on with string  
Remove the latter

Use a jar or large tube of freshly prepared thioglycollate medium, preferably still warm from the steriliser but not above 40°C. There will be no coloured region.

Using a large bulb and pipette draw up enough broth to almost fill the bottle and transfer it carefully. To ensure gentle delivery keep the tip of the pipette beneath the surface of the ejected liquid. The very slight oxygenation that takes place is corrected by the reducing agents in the medium. If the volume to be transferred is large the type of bulb used for handling radioactive and other dangerous liquids is useful.

Substitute a dry heat sterilised metal test tube cap for the paper cover and incubate. The thioglycollate is used as a joint medium.

##### (b) *Ampoules*

Aseptically open the ampoules and cover them with Durham tubes.

Use fresh thioglycollate as above but transfer it with a syringe and fill each ampoule up to the constriction. Incubate, covered with the Durham tubes or sealed by fusion of glass. With the latter method it is better to carry out the aerobic and anaerobic tests separately, fill the aerobic ampoules to  $\frac{1}{3}$  capacity only, using nutrient broth, and shake at intervals during incubation to detach organisms from the surfaces above the liquid. A suitable positive control will confirm that there is sufficient air in the sealed ampoule for the growth of aerobes.

#### 2 Syringes

There are two approaches to this problem.

(a) The assumption can be made that since the inside of the syringe is the part least easily reached by a sterilising agent its condition can be used as an indicator of the condition of the rest.

Unless the syringe already has a needle attached, aseptically fix on one that is known to be sterile. Draw nutrient broth into the barrel, agitate the syringe gently to detach organisms from the surfaces, and distribute the contents equally into separate aerobic and anaerobic media or into the top and bottom of a joint medium.

A possible objection to this method is that organisms may not be removed from the syringe by gentle shaking.

(b) The complete syringe can be tested. This overcomes the disadvantage of method (a) and, since it tests the outside of the syringe, provides a better check on unsatisfactory packaging.

Dismantle the syringe and put it into a joint medium, preferably using a tall jar in which the parts will stand upright, i.e. with different areas of their surfaces at different oxidation-reduction potentials. This method is satisfactory for all-glass and plastic syringes but thioglycollate sometimes reacts with the metal parts of glass-metal types, causing the medium to darken and become less suitable for bacterial growth. One solution is to put the barrel into nutrient broth and the plunger into cooked meat.

The unpacking and handling of a syringe is scarcely less hazardous from the point of view of accidental contamination than the manipulation of a surgical dressing and, therefore, when large batches are under test a design similar to that for dressings is desirable.

### 3 Disposable Transfusion and Infusion Assemblies

The U S P XVI applies the following test to representative samples.

The lumens of each of not less than 10 assemblies taken from various levels in the steriliser chamber are flushed with 40 ml of Fluid Thioglycollate Medium, which is collected in empty sterile tubes and incubated. The needles are either treated similarly, using 10 ml volumes of the medium, or are incubated in the medium.

### Miscellaneous Tests

diagnostic preparations) or destroyed in (suspensions) the final products. Usually three methods are available.

(a) Sterility tests in which the media and incubation conditions are tailored to the particular pathogen.

### 1. IMMUNOLOGICAL PREPARATIONS

Living pathogenic bacteria are used in the production of many immunological preparations (see Cooper and Gunn, 1957b) and it is imperative to show that they have been removed from (toxins, toxoids and

used in the preparation. This is an advantage not available in normal sterility testing.

(b) Conventional sterility tests

(c) Toxicity tests Most immunological preparations are tested for toxicity by the injection of massive doses into susceptible animals. If living pathogens are present disease will result unless the infection is so slight that the animal's defences can overcome it.

When, as is usual, all these tests are performed and the sterilisation and aseptic processes are carefully controlled there is no risk of pathogens in the issued products.

Bacterial sterility tests can be applied to living viral vaccines such as yellow fever vaccine because viruses can multiply only in the environment of a living cell and, therefore, will not grow in a bacteriological test medium. However, smallpox vaccine, which contains living cowpox virus, cannot be subjected to these tests because, when it is made by using free-living animals, complete exclusion of bacterial contaminants is impracticable, and although most are destroyed in the preparation of the vaccine sterility cannot be guaranteed. The *British Pharmacopœia* tolerates not more than 1,000 viable organisms (i.e. bacteria) per ml, and these would vitiate a sterility test. Nevertheless pathogens cannot be allowed in this population and tests are directed to exclude them.

For many of the type (a) tests agar and blood plates are needed. They are prepared as follows.

#### Agar Plates

When only a few plates are required the nutrient agar is most conveniently supplied in 1-oz narrow-mouthed McCartney bottles. It is melted in a boiling water bath and then transferred to a bath controlled at 45°C until needed.

Assuming the petri dishes have been sterilised singly or in groups of three (see chapter 22), they are unwrapped with the lids uppermost. (The space between lid and base can be felt through the paper.) This prevents airborne organisms from falling into the rather large space between the side of the dish and the flange of the lid.

The base of each dish is labelled distinctly and distinctively. The base is used so that if the lids of several dishes become interchanged the identification remains attached to the work to which it refers.

One bottle is removed from the water bath. It is inadvisable to remove many because some may solidify before they can be poured. The outside is wiped because the water of the bath will not be

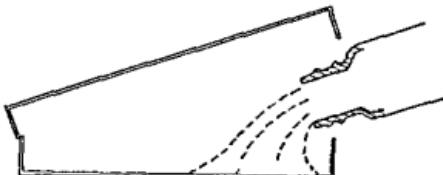


Fig. 25.5 POURING AN AGAR PLATE

sterile at 45°C and, therefore, drops falling into the plate during pouring may cause contamination.

The bottle is held nearly horizontal in the R.H., the cap is removed with the L.H. and placed on the lid of the dish (this is quicker than putting it on the bench) and the lid is immediately raised at one side just enough to insert and tilt the bottle neck, but without exposing the base of the dish to aerial bacteria (Fig. 25.5). The agar is poured, the bottle removed and the lid gently lowered.

When organisms have to be mixed with the agar they are pipetted, with protection from the lid, into the dish before the medium is poured. All the precautions for handling live organisms must be rigidly observed. Immediately after addition of the agar the suspension is evenly distributed by a series of diagonal and circular movements carried out in the order shown in Fig. 25.6, and each repeated five times before continuing to the next. They must not be too vigorous or the medium will be thrown over the edge of the dish.

Agar is used at 45°C, primarily because at this temperature it does not harm living bacteria. However, even if organisms are not to be inoculated until the medium is solid (i.e. by spreading or streaking) the agar should not be poured straight from the boiling water bath because heavy condensation collects on the inside of the lid of the dish and may fall on the surface of the plate causing spreading of the

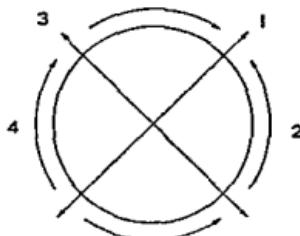
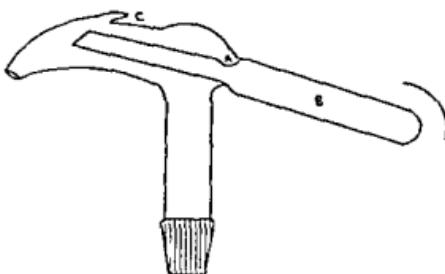


Fig. 25.6 MIXING THE CONTENTS OF A PETRI DISH



*Fig. 257 KIPPS MEASURE  
A—hole inlet to B B—measuring chamber C—air inlet*

colonies and consequent difficulty in counting and identification. Also, the dishes may be cracked by contact with the hot bottles.

Large numbers of plates are more conveniently poured with a Kipps measure (Fig. 257) attached to a flask of the medium. The chamber is filled by tipping the flask in the direction of the arrow, on returning the apparatus to the vertical, excess agar runs back into the flask, leaving the calibrated volume in the chamber, this is poured out of the spout into the dish. The agar must be considerably warmer than 45°C or it will gel at the outlet before the flask is empty. The minimum temperature suitable for a particular number of plates is soon found by experience.

#### *Blood Agar Plates (see chapter 19)*

The only important difference from plain plates is that blood is added to the bottle just before pouring. The agar cannot be used at 45°C because the addition of the cool blood makes it solidify immediately, but it must not be too hot or the blood proteins will precipitate. A temperature of 55°C is suitable.

First, the blood cells are evenly distributed by rotating the container. If this is omitted plates with different cell contents and, therefore, different colours will be obtained.

Then the blood is measured with a pipette. Although the volume is not critical, so long as it is approximately 5 to 10 per cent of the final medium, plates of equal composition are necessary for critical work. The transference is carried out in the same way as a tube to tube aseptic transfer (q.v.).

After replacement of the closures the cap on the agar bottle is tightened and the container inverted several times until the mixture is homogenous. This method is more efficient than rotating the bottle.

Finally, the agar is poured into the dish taking

care to leave behind in the bottle the froth caused by shaking.

Blood pipettes should not be put into lysol after use because this causes the blood to swell, adhere and precipitate, and subsequent removal of the coagulum by washing is almost impossible.

Inoculated plates should be incubated inverted to prevent condensate produced inside the lid during incubation from dropping on to the medium with the results mentioned above.

## 2 IMPLANTS

These are small tablets intended for depot medication. There are two examples in the B.P.—Deoxycortone acetate and Testosterone. They are implanted intramuscularly or subcutaneously by a minor surgical operation and, therefore, must be sterile.

To ensure very slow solution in the tissues they are

- (a) made from water-insoluble compounds,
- (b) heavily compressed or fused,
- (c) free from all the adjuncts (e.g. disintegrants) used in normal manufacture.

Consequently, they do not dissolve when added to aqueous sterility test media and must be crushed before inoculation to expose the interior. The resulting insoluble powder is treated as such.

Separate implants must be used for the aerobic and anaerobic tests.

## FILTRATION TECHNIQUES IN STERILITY TESTING

In 1946 Davies and Fishburn pointed out several drawbacks in the official method of sterility testing. They included—

- 1 The large amount of work necessary to discover which medicaments are inhibitory and to find suitable neutralising agents for these and for preservatives.
- 2 The loss of sensitivity in tests in which high dilution is used to overcome inhibitory effects.

They recommended an entirely different technique. In this, contaminants are removed from the sample by filtration through a sterile bacteria proof filter pad. Then bactericides and inhibitory medicaments are removed from the organisms and filter by washing with a sterile solvent and, finally, the whole of the pad is incubated in a suitable culture medium.

They used an asbestos-cellulose filter pad (a 3-cm Grade SB Ford Sterimat) which was mounted in a special holder in which the pad was not exposed to aerial contamination, i.e. the top of the holder was

plugged and the edge of the pad was enclosed (contrast the holder illustrated in Fig. 26.8b)

The following procedure was suggested

Aseptically filter half the solution

Wash the pad with three 10-ml volumes of sterile distilled water

Transfer the pad to aerobic broth

Repeat the procedure with the rest of the injection and a fresh pad, transfer this to anaerobic broth

Incubate in the usual way

Solids for injection were first dissolved in sufficient sterile distilled water to produce the strength normally used parenterally (Stronger solutions of antibacterial medicaments might destroy contaminants)

The technique was also recommended for oils as an alternative to the normal shaking method. The sample was shaken with an equal volume of sterile light petroleum until the mixture was homogeneous. Then the procedure for aqueous solutions was followed except that light petroleum was used for washing. Previous investigations had indicated that the solvent was bacteriologically inert.

Sykes and Hooper (1959) developed a similar method in which the asbestos pad was replaced by a membrane filter (see chapter 26). The chief advantages are—

1 The membranes are so thin that retention of inhibitory substances is very small. Asbestos-cellulose pads are relatively thick and fibrous and, therefore, may absorb and retain sufficient inhibitor to cause bacteriostasis in the culture medium.

2 Quicker filtration. Sykes found that a 20 per cent solution of streptomycin took 68 min and 60 sec to filter through asbestos and membrane filters respectively.

3 Oils pass through easily and quite quickly and there is no need to dissolve them in an organic solvent first. It is a criticism of Davies and Fishburn's method that the light petroleum may adversely affect the viability of some organisms.

#### ADVANTAGES OF FILTRATION TECHNIQUES

- Wide application. They can be used for aqueous and oily solutions and solids for injection—but not for suspensions.
- There is no limit to the volume that can be tested with one pad. Therefore, the methods are applicable to the testing of poorly soluble solids.
- Where, when the conventional method is used, a large volume of medium is necessary this can be reduced to the volume needed for incubating the pad.
- They are applicable to substances for which no

satisfactory inactivators are known, e.g. many antibiotics.

- Some strongly adsorbed antibacterial agents such as the mercurials and quaternary ammonium compounds, can be inactivated on the filter by treatment with the appropriate neutralising solution.
- Subculturing is often eliminated, e.g. for oils and oily preparations and substances that, like the barbiturates, give precipitates in broth.

#### DISADVANTAGES OF FILTRATION TECHNIQUES

- Even with membrane filters the possibility of adsorption of sufficient medicament to vitiate the test cannot be discounted entirely.
- Highly skilled staff and exceptionally good aseptic technique are necessary. An asepsis room is essential unless a sealed screen of the type developed by Royce and Sykes (1955) is available. The latter, because the risk of accidental contamination is so low, overcomes an important criticism of the filtration method—that it is illogical to use in sterility testing a technique (bacterial filtration) that is itself not considered sufficiently reliable for its products to be issued without sterility tests on every batch.

#### USE IN THE BRITISH PHARMACOPOEIA

The *British Pharmacopoeia* recommends the filtration technique, using membrane filters, for the sterility testing of antibiotics other than benzathine, benzyl, and procaine penicillin.

A saline solution containing approximately 0.1 per cent of the antibiotic is filtered through a 5-cm membrane, previously moistened with saline to reduce retention of the antibiotic (Lightbown, 1962), and having a maximum pore diameter of 0.75  $\mu$ . After washing with a litre of saline solution or other suitable solvent the membrane is halved and one half is tested for aerobic and the other for anaerobic organisms. Alternatively, the whole disc may be incubated in a joint medium.

The antibiotics tested in this way include bacitracin, methicillin sodium, oxytetracycline hydrochloride, polymyxin B sulphate, streptomycin sulphate, tetracycline hydrochloride, vancomycin hydrochloride and viomycin sulphate.

(See also Desbordes and Ninard, 1962 and Millipore Corporation, 1963.)

#### TESTS FOR MYCOTIC STERILITY

A test for mycotic sterility, i.e. freedom from moulds and yeasts, is prescribed in the U.S.P. XVI for all preparations that have not been heated to at least

100°C for 15 min. The WHO Report recommends similar tests for biological products.

The general principles are the same as for bacterial tests. The main differences (which have been explained in chapter 19) are—

**The Medium** Only aerobic media are required. The U.S.P. specifies Fluid Sabouraud's. All the media in the WHO Report are solid, i.e. Malt, Malt Extract and Sabouraud's agars.

**The Surface Area Exposed during Incubation** The U.S.P. recommends a large surface area. Containers should be incubated on their sides or sloped to as near to horizontal as possible.

#### *The Incubation Time and Temperature*

22° to 25°C for at least 10 days (U.S.P.)

20° to 25°C for 14 days (WHO Report)

**The Organism Used in the Positive Controls** The U.S.P. and WHO Report recommend the yeast *Candida albicans* and the former advises a 1 in 1,000 dilution of a 24 to 48 hr culture. (See also Pittman and Feeley, 1962 and Proom, 1962.)

## TESTS FOR VIRAL STERILITY

The WHO Report contains the first attempt to give guidance on tests for viral sterility. These are necessary for biological products such as viral and rickettsial vaccines in the preparation of which the production organism is grown in a living cell system, i.e. in free living animals, fertile eggs or tissue cultures. In these environments contaminating viruses may find suitable conditions for multiplication.

The difficulty of detecting viral contamination is reflected by the great emphasis in the Report on the prevention of contamination. Reference should be made to the Report for full details but the recommendations include—

1. Separate isolated areas for the manufacture of each vaccine.
2. Precautions to prevent the dissemination of viruses to other areas via the air conditioning system or the drains.
3. Regular checks on the air conditioning and its filters.
4. Autoclaving of all contaminated material before it leaves the area, even if it is going to washing facilities reserved for that area.
5. A separate area, to which only heat-sterilised materials are admitted, for the preparation of media sterilised by filtration.
6. Double-ended autoclaves as the only connexion between sterile and unsterile areas.
7. A separate area for the preparation of tissue

cultures including a special room for the aseptic removal of the monkey kidneys that are often used in these cultures.

8. A separate area for filling each live virus vaccine into its containers.

Two major problems in the testing of preparations for viral sterility are—

- (a) Living viruses must be detected by some effect that they produce on living cells, e.g.
  - (i) The death of an animal.
  - (ii) Lesions (wounds) on the skin of an animal (e.g. the pustules of smallpox).
  - (iii) Lesions on the chorioallantoic membrane of a living egg.
  - (iv) Changes in the appearance of the thin layers of living cells used in tissue cultures.
  - (v) Agglutination of red blood cells.

Highly trained personnel are necessary to design, perform and interpret these tests.

- (b) Sometimes it is necessary to test for foreign viruses in preparations containing living viruses.

If possible, a living cell system is used in which the virus of the vaccine will not grow. Alternatively, it may be possible to destroy the product virus with the corresponding antiserum or to alter the growth conditions (e.g. incubation temperature or degree of aeration) so that its growth is prevented. This problem is particularly difficult when preparations containing attenuated (weakened) strains have to be tested for freedom from the virulent (dangerous) strains of the same organisms, as in the oral poliomyelitis vaccine.

Tests are performed at several stages—

- (a) On the seed virus, i.e. the virus that will be used to produce the vaccine.
- (b) On the growth tissue, i.e. the tissue culture in which the seed virus is grown.
- (c) On the final vaccine.

They are carried out by inoculating suitable living cell systems with the seed virus (a), the fluid in which the tissue culture has been growing (b) or the vaccine (c). Virus inhibitors, such as serum, must be absent.

Tests for bacterial and mycotic sterility are also necessary because the highly nutritive tissues and media used in virus vaccine production provide excellent conditions for the growth of other micro-organisms.

Further consideration of this subject is impracticable without a detailed explanation of virus vaccine

production but this short account will indicate its importance and complexity

## REFERENCES

- 1 ALLPORT, N L (1952) Cetrimide and benzalkonium chloride *Pharm J* 169, 435-437
- 2 BERRY, H and JENSEN, V G (1951) On a sterility test for neo- and sulpharsphenamine *Acta Pharm Intern* 2, 221-234
- 3 BOOTH, T G (1955) Factors affecting tests for sterility of derivatives of barbituric acid *J Pharm Pharmacol* 7, 268-275
- 4 BRITISH STANDARD 3286 1960 *Method for the laboratory evaluation of quaternary ammonium compounds* British Standards, Institution, London
- 5 BRYCE, D M (1956) The design and interpretation of sterility tests *J Pharm Pharmacol* 8, 561-572
- 6 BULLOCK, K and BOOTH, N H (1953) Bacterial survival in systems of low moisture content Part V Comparison of tests for sterility of oils *J Pharm Pharmacol* 5, 757-771
- 7 BULLOCK, K and KEEPE, W G (1951) Bacterial survival in systems of low moisture content Part III Bacteria in fixed oils and fats Section I Viable counts of micro organisms in fixed oils and fats *J Pharm Pharmacol* 3, 700-716
- 8 COOK, A M (1961) Methods and media used in sterility tests *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London 185-189
- 9 COOK, A M and STEEL, K J (1960) The antagonism of the antibacterial action of mercuric chloride *J Pharm Pharmacol* 12, 219-226
- 10 COOPER, J W and GUNN, C (1957a) *Tutorial Pharmacy* 5th Ed Pitman, London 669
- 11 COOPER, J W and GUNN, C (1957b) ibid 554-572
- 12 COULTHARD, C E (1935) Sterilisation by dry heat at 150°C with special reference to oils *Quart J Pharm*, 8, 90-93
- 13 DAVIES, G E and FISHBURN, A G (1946) The testing of drugs and pharmaceutical preparations for sterility A new technique *Quart J Pharm* 19, 365-372
- 14 DAVIES, O L and FISHBURN, A G (1948) Sampling for sterility tests *Pharm J* 160, 184-185
- 15 DELUCA, P P and KOSTENBAUDER, H B (1960) Inactivation of preservatives with macromolecules Binding of quaternary ammonium compounds by non ionic agents *J Amer pharm Ass, Sci Ed* 49, 430-437
- 16 DESBORDES, J and NINARD, B (1962) Le point de vue du contrôleur et de l'expert dans l'essai de stérilité des produits utilisés en médecine humaine *Proceedings of the 7th International Congress for Microbiological Standardisation* Livingstone, Edinburgh 177-206
- 17 DIFCO MANUAL (1953) *Difco Manual of Dehydrated Culture Media and Reagents* 9th Ed Difco Laboratories Inc., Detroit 1, U S A
- 18 HUNTER, C L F, HARBORD, P E and RIDDETT, D J (1961) Packaging papers as bacterial barriers *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London 166-172
- 19 JACOBS, S E and HARRIS, N D (1961) The effect of modifications in the counting medium on the viability and growth of bacteria damaged by phenols *J Appl Bact* 24, 172-181
- 20 JEZKOVA, Z (1960) A comparative study of sterility test media in the transfusion service *Appl Microbiol* 8, 274-277
- 21 KELSEY, J C (1961) Acceptable standards for surgical materials *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London 203-207
- 22 KLARMANN, E G (1950) The role of antagonisms in the evaluation of antiseptics *Ann N Y Acad Sci* 53, Art 1 123-146
- 23 KNUDSEN, L F (1949) Sample size of parenteral solutions for sterility testing *J Amer pharm Ass, Sci Ed* 38, 332-337
- 24 LAWRENCE, C A (1948) Inactivation of the germicidal activity of quaternary ammonium compounds *J Amer pharm Ass, Sci Ed* 37, 57-61
- 25 LAWRENCE, C A (1950) Mechanism of action and neutralising agents for surface-active materials upon micro-organisms *Ann N Y Acad Sci* 53, Art 1 66-75
- 26 LIGHTBOWN, J W (1962) The sterility testing of antibiotics *Proceedings of the 7th International Congress for Microbiological standardisation* Livingstone, Edinburgh 220-225
- 27 MICHAELS, I (1950) The use of inhibitory agents in investigations of antibacterial activity *Pharm J* 164, 263-264, 302-303
- 28 MILLIPORE CORPORATION (1963) *Sterilising filtration and sterility testing* Millipore Filter Corporation, Bedford, Mass., U S A
- 29 PITTMAN, M (1946) A study of fluid thioglycollate medium for the sterility test *J Bact* 51, 19-32
- 30 PITTMAN, M and FEELY, J C (1962) Sterility testing Detection of fungal and yeast contamination in biological preparations *Proceedings of the 7th International Congress for Microbiological Standardisation* Livingstone, Edinburgh 207-214

- 31 POLLOCK, M. R. (1957) A simple method for the production of high titre penicillinase *J Pharm Pharmacol* 9, 609-611
- 32 PROOM, H. (1962) Sterility test regulations and the manufacturer *Proceedings of the 7th International Congress for Microbiological Standardisation* Livingstone, Edinburgh. 169-176
- 33 QUILSTO, R., GIBBY, I. W. and FOTER, M. J. (1946) A neutralising medium for evaluating the germicidal action of the quaternary ammonium compounds. *Amer J Pharm* 118, 320-323
- 34 ROYCE, A. and SYKES, G. (1955) A new approach to sterility testing *J Pharm Pharmacol* 7, 1046-1052
- 35 RYGIEL, W. V. (1961) Use of tyloxapol as an emulsifier in a sterility test for oils *J Pharm Sci* 50, 703-707
- 36 SAVAGE, R. M. (1940) Sterility tests on surgical dressings *Quart J Pharm* 13, 237-251
- 37 SAVAGE, R. M. (1942) The sterilisation of paraffin surgical dressings *Brit Med J* 1, 472-474
- 38 SAVAGE, R. M. (1961) Interpreting the results of sterility tests *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London. 190-198
- 39 STEEL, K. J. (1960) A note on the reaction of mercuric chloride with bacterial -SH groups *J Pharm Pharmacol* 12, 59-61
- 40 SYKES, G. (1956) The technique of sterility testing *J Pharm. Pharmacol* 8, 573-588
- 41 SYKES, G. and HOOPER, M. C. (1959) A note on the use of membrane filters in sterility testing *J Pharm. Pharmacol* 11, 235T-239T
- 42 SYKES, G., ROYCE, A. and HUGO, W. B. (1952) A sterility test for Neoarsphenamine B.P. and Sulpharsphenamine B.P. for injection. *J Pharm. Pharmacol* 4, 366-374
- 43 TATTERSALL, K. (1961) Control of sterility in a manufacturing process *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London. 198-203
- 44 THERAPEUTIC SUBSTANCES REGULATIONS (1963) *Statutory Instruments 1963 No 1450 Therapeutic Substances* Her Majesty's Stationery Office, London.
- 45 WILLIAMSON, G. M. (1957) The mode of action of streptomycin. *J Pharm Pharmacol* 9, 433-445
- 46 WORLD HEALTH ORGANISATION (1960) *Wld Hlth Org techn Rep Ser* 1960, 200 *Requirements for Biological Substances b General requirements for the sterility of biological substances Report of a study group* Her Majesty's Stationery Office, London.
- 47 YARLETT, M. A., GERSHENFELD, L. and MCCLENAHAN, W. S. (1954) Petrolatum gauze I Methods of sterility testing *Drug Standards* 22, 205-210

## Aseptic Processing

**ASEPTIC** processing is concerned with the preparation of those sterile products that cannot be subjected to a terminal heating process because the medicaments they contain are thermolabile (see the beginning of chapter 24). In many cases it involves sterility tests on samples from each batch.

The most important example is Sterilisation by

### I. STERILISATION BY FILTRATION

Filtration through a bacteria proof filter is a suitable method for the sterilisation of injections containing thermolabile medicaments. However, there is an important limitation—the medicament must be stable in solution. Many thermolabile substances are not and, therefore, filtration is less widely used for this type of preparation than might be expected. This is confirmed by a comparison of the 'Filtration' and 'Aseptically prepared' sections of the classified list of official injections in Appendix 4.

Nevertheless, in industry, filtration is sometimes preferred to a heating process for the sterilisation of *thermostable* injections. Mother liquors from which

Filtration but there are many others including—

The packaging of thermolabile solids for injection  
The preparation of injections from such solids  
The preparation of sterile dusting powders containing thermolabile medicaments  
The preparation of eye ointments

thermolabile solids will be crystallised or precipitated subsequently are also sterilised in this way

The process involves four stages—

- 1 Filtration of the solution through a bacteria-proof filter
- 2 Aseptic distribution of the filtered solution into previously sterilised containers
- 3 Aseptic closure of the containers
- 4 Testing of samples for sterility

Sterility tests are obligatory for injections sterilised by filtration, and until they have been passed no part of the batch may be issued, except in an emergency (see chapter 25).

#### Bacteria-proof Filtration Media

There are four classes—

- 1 *Sintered ceramics*—made from finely ground porcelain or from diatomaceous earth
- 2 *Fibrous pads*—containing asbestos and wood cellulose
- 3 *Sintered glass*—made from borosilicate glass
- 4 *Microporous plastics*—prepared from cellulose esters, particularly the acetate and nitrate

Sintered media are made by heating tightly packed particles of the material in a suitable mould at a temperature below their melting point. The particles become welded together at the points of contact, which increase in area but do not grow large enough

to obliterate the inter-particle spaces. Therefore, a porous mass results in which the porosity depends on the size of particle used.

Sintered ceramic filters are in the form of hollow cylinders, usually closed at one end and called candles. The other media are used in the form of discs. Candles present a larger surface to the filtering liquid but retain a correspondingly greater volume inside the matrix at the end of the process.

Fibrous pads and microporous plastics are used once and then discarded.

All types are available in a range of porosities but only the finest are suitable for removing bacteria. (Microporous plastics are exceptional in that

practically all grades are bacteria-proof). Types with large pores are intended for clarification and are often used for this purpose in the preparation of sterile solutions, e.g. sintered glass, grades 3 and 4 (see chapter 23).

Bacteria proof filters of micro-porous plastics are thin membranes, very tough when wet, with pores small enough to sieve out bacteria from liquids or air. The other media must be fairly thick, for strength, and they filter much too slowly if their pores are small enough to retain bacteria by mechanical sieving, therefore, grades with pores larger than many bacteria are used, and they function satisfactorily because retention is assisted by factors other than inability of organisms to pass into the pores (see 'Mechanisms of Bacterial Filtration').

Usually a sintered medium is bacteria-proof if the maximum pore diameter does not exceed  $2.5\text{ }\mu$ , because, generally, such a filter retains bacteria that are not smaller than about  $\frac{1}{3}$  of the maximum pore size, i.e. all common species. However, the efficiency of this type of medium must always be confirmed before its initial use and at regular intervals during its lifetime (see 'Testing of Filters').

Filtration through bacteria proof media is very slow or negligible under atmospheric pressure and must be accelerated by applying pressure to the liquid or vacuum to the clean side of the filter.

Sykes (1958a) has summarised the features of a satisfactory medium—

- 1 The porosity of the material must be sufficient to allow an adequate filtration rate for the job in hand, without constant blockage.
- 2 It must yield nothing to the solution and must remove nothing but organisms and insoluble material.
- 3 Its properties must not alter during moist or dry heat sterilisation.
- 4 It must be easily assembled into, or form an integral part of, a leak-proof filtration unit.
- 5 If not disposable, it must be easy to clean by a method that does not alter its porosity.
- 6 If it requires fitting into a metal holder (this applies to disposable media) the metal must not affect the solution being filtered.

It is difficult to satisfy all these requirements as the following consideration of the individual types of media will show.

#### A SINTERED CERAMICS

##### 1 Unglazed Porcelain

Early filters of this type, such as the Pasteur-Chamberland (introduced by and named after the

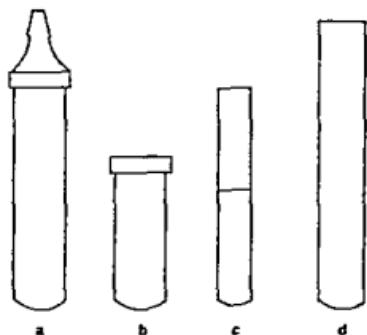


Fig. 261 UNGLAZED PORCELAIN FILTERS

famous bacteriologist and one of his colleagues) were produced by sintering a mixture of kaolin (China clay) and quartz sand, but modern forms are made from finely ground unglazed porcelain. Examples are the Doulton 'Pasteur', manufactured by the Royal Doulton potteries in this country, and 'Selas' filters (Martin and Cook, 1961) widely used in America.

Stock sizes of Doulton candles range from 55 mm long by 10 mm diameter to 205 mm long and 25 mm diameter. Their walls are about 3 mm thick. Some sizes have glazed porcelain mountings (Fig. 261a,b) to facilitate incorporation into filtration units. These mounts are attached by glazed joints to avoid the use of cement which is a potential source of weakness because it may become cracked or otherwise damaged, as a result of careless sterilisation or cleaning, and provide channels through which contamination may occur during use. Unmounted candles with a glazed top (Fig. 261c) are also available. A disadvantage of filters with glazed attachments is the difficulty of cleansing residual solution from beneath the glazing or within the mount. Consequently, entirely unglazed and unmounted types (Fig. 261d) should be used whenever possible. The maximum pore size is usually between 2 and  $2.5\text{ }\mu$ . Some of the characteristics of this type of filter are indicated by the following data—

1 With a pressure difference of  $5\text{ lb/in.}^2$  between the inner and outer faces, a candle 75 mm long by 15 mm diameter and having a maximum pore diameter of  $2\text{ }\mu$  will pass 550 ml of water/hr until it begins to clog.

2 An increase of approximately 1 mm in wall thickness decreases the flow rate by about 20 per cent.

3 The flow rate through a filter of maximum pore size  $0.75\ \mu$  is only about 12 per cent of the rate when the maximum pore is  $2\ \mu$ . For this reason sintered materials are not made in very small pore sizes.

#### *Advantages*

- 1 These filters are robust. They stand up well to handling, to filtration pressures and vacua and to drastic cleaning methods.
- 2 Only small amounts of medicaments are adsorbed from solutions undergoing filtration.

#### *Disadvantages*

- 1 They are less easy than most other types to fit into filtration units.
- 2 They become blocked rather quickly with micro-organisms and foreign particles.
- 3 Cleaning can be complicated—

#### CLEANING

##### 1 New Filters

These are assembled in a filtration unit and

- (a) Washed free from the dust of manufacture by drawing through a large volume of water.
- (b) Treated with hot Dilute Hydrochloric Acid B.P., to remove adsorbed impurities.
- (c) Freed from acid by thoroughly washing with tap and then pyrogenic water.
- (d) Dried, from cold, in a drying oven. If filters are stored wet pyrogens may be produced within the pores.

##### 2 Used Filters

- (a) Immediately soaked in distilled water. They must not be allowed to dry because the resulting deposits are very hard to dislodge.
- (b) Scrubbed inside and out with a stiff brush. It is an advantage to filter on to the outside of a candle (see (2) in Fig. 26.6) because it is difficult to examine the inside during cleaning.
- (c) Flushed with pyrogenic water in the opposite direction from use to dislodge remaining loosened surface deposits.
- (d) Dried.

More drastic treatments are necessary when reduced flow indicates serious blockage of the pores.

(i) *Chemical* Deposits are removed with oxidising agents such as hypochlorite, acid permanganate (0.5 per cent) followed by sodium thiosulphate (5 per cent), or strong acids (e.g. nitric). Chromic acid cleaning solution is unsuitable because chromic ions are strongly adsorbed by the filter and may not be completely removed by the subsequent washing, with

the result that the medicament may be oxidised when the filter is next used. For filters with mounts fixed with cement it is necessary to confirm that the latter will withstand the treatment proposed.

(ii) *Heat* The filter is dried thoroughly and put into a cold muffle furnace. The temperature is very slowly raised to  $600^\circ\text{C}$  and maintained for several hours (e.g. 5, including heating-up). The candle is allowed to cool in the furnace and afterwards is well flushed to remove residues, and finally dried. The method is unsuitable for filters with mounts fixed with cement.

Each of these procedures may affect the porosity of the medium and this should be checked (see 'Testing') before the candle is re-used.

Sometimes unglazed porcelain filters are used in the manufacture of immunological products. These often contain high concentrations of sticky heat-coagulable protein and, therefore, cleaning can be particularly difficult. One method is to incubate the filter overnight at  $37^\circ\text{C}$  in alkaline trypsin solution—the enzyme digests the proteins to simple water-soluble degradation products that can be flushed away.

#### 2 Kieselguhr

These candles contain a high proportion of kieselguhr, a purified siliceous earth consisting mainly of diatoms, hence the fineness of the pores. Media of different pore sizes are produced by including appropriate amounts of other substances such as asbestos and calcium sulphate. The ingredients are made into a paste which is heated to sintering point in suitable moulds.

The earliest and, probably, still the most important commercial type is the German Berkfeld filter. Another is the American Mandler. In this country the Doulton range of industrial filters includes kieselguhr types but bacteria proof grades are no longer listed because sintered ceramics are less popular than some alternative media. The demand can usually be satisfied with unglazed porcelain, which has fewer disadvantages than kieselguhr.

Kieselguhr candles are thicker than unglazed porcelain types and normally have glazed porcelain or metal (Fig. 26.2) mounts attached by cement. Preferably the metal should be stainless steel.



Fig. 26.2 KIESELGUHR FILTER (WITH METAL MOUNT)

***Disadvantages***

1. On the whole they are less robust than unglazed porcelain filters. This is particularly true of small sizes. They should be handled gently, cleaned with a soft brush, protected from sharp pressure changes and allowed to cool completely before removal from the autoclave.

2. Often, appreciable adsorption of medicaments occurs. They are the least satisfactory filters in this respect.

3. After use for filtering oils they are particularly susceptible to breakage and are very difficult to clean (Avis and Gershenson, 1955).

**CLEANING**

The choice of drastic methods is limited. Hypochlorites or permanganate can be used but strong acids and heat treatment should be avoided.

**B FIBROUS PADS**

These are soft pads about 3 mm thick, usually round but occasionally square, consisting largely of compressed asbestos. Used alone, asbestos becomes tightly compacted and the filtration rate falls considerably. Therefore, it is usually blended with wood cellulose which keeps the porosity high. Other substances may be added but are trade secrets.

They were invented in Germany and marketed under the trade name of Seitz. The bacteria proof grade is coded 'EK'. An equivalent is now made in this country—the Ford Sterimat, the 'SB' quality of which is bacteria proof.

Sterimats are obtainable in discs 3, 6, 6 and 14 cm in diameter or in large square sheets. The latter and the 14 cm discs can be used in filter presses. The medium is spongy and fragile when wet and during filtration must be supported on a perforated metal or glass disc in a suitable holder (Fig. 26.8). A 6 cm disc will filter about 200 ml of water/hr when the pressure difference between the faces is 5 lb/in<sup>2</sup>.

***Advantages***

1. A new pad is used each time. There is no risk of contaminating the filtrate with either incompletely removed residue from a previous filtration or traces of cleaning agents. The pads are inexpensive. The holders are easy to dismantle and clean.

2. They clog less easily than other media.

3. They are more suitable than ceramics or glass for viscous solutions.

4. They can be used in the filtration method of sterility testing.

***Disadvantages***

1. Alkali may be given to the filtrate (Browne, 1942), which may be sufficient to precipitate alkaloids from solutions of their salts and affect the stability of alkali sensitive medicament such as insulin, the posterior pituitary hormones, adrenaline, apomorphine and aneurine. Highly buffered solutions, e.g. culture media, are unaffected.

When adverse effects are probable, N/100 hydrochloric acid should be drawn through the pad (20 ml is usually more than adequate for the 6 cm size) and followed by sufficient pyrogenic water to give a neutral solution. This is done before sterilisation.

2. Loose fibres may separate and contaminate the filtrate. This can be remedied by attaching a tiny grade 3 or 4 sintered glass clarifying filter to the stem of the holder (Fig. 26.3).

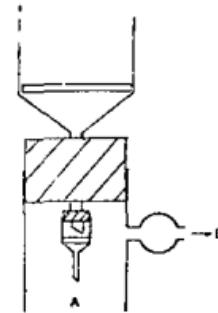


Fig. 26.3 USE OF SMALL SINTERED GLASS FILTER TO RETAIN FIBRES FROM ASBESTOS CELLULOSE PAD

A—Grade 3 or 4 sintered glass filter  
B—to vacuum pump

3. Significant adsorption of medicaments often occurs and can cause serious loss of strength in solutions of small volume. It is particularly marked with basic dyes and enzymes. With large volumes adsorption will take place from the first part of the filtrate and, if the volume affected is determined by assay, it can be discarded in subsequent filtrations.

4. Sharp pressure changes may break the wet pads and cause contamination of the filtrate with unfiltered, unsterile solution. There should be a buffer flask between the pump and the filter so that pressure is not applied directly to the pad. This is important even when a hand pump is used. Also, the pressure or vacuum should be released gradually.

5. They are unsuitable for strongly alcoholic solutions (see 'Mechanisms of Bacterial Filtration').

### C SINTERED (FRITTED) GLASS

This medium is popular for sterilising small volumes. It is manufactured by powdering high grade borosilicate glass in a ball mill (borosilicate balls are used), separating the finest particles by air elutriation and sintering appropriate sizes into discs in suitable moulds. The discs are fused into Buchner type (usually) or pipe line glass holders (Smith, 1944).

Grade 5 (maximum pore size not more than  $2\text{ }\mu$  — see B.S. 1752 1963) is used for removing bacteria. Filtration is slow through filters in which the maximum pore is less than  $1.5\text{ }\mu$ . This is also the case when the disc is thick, but because thin discs cannot withstand the pressures and vacua of bacterial filtration it is customary to support a thin layer of grade 5 on a thicker layer of grade 3 (a clarifying grade). Consequently, bacteria proof sintered glass filters are usually known as 5 on 3 (Fig. 26.4).

#### *Advantages*

- 1 If properly cleaned they yield nothing to the filtrate.
- 2 There is very little adsorption of medicaments.
- 3 The volume of filtrate retained in the medium is less than with ceramic types.
- 4 If desired, the holders can be fused into glass filtration units, thus reducing the number of possible leakage points and facilitating cleaning (Sykes, 1958a).

#### *Disadvantages*

- 1 The medium is unsuitable for large volume filtrations because large discs are mechanically weak.
- 2 The filters and, particularly, their holders are easily broken and replacement is costly.

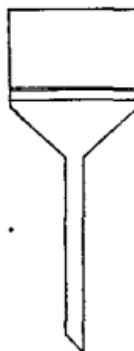


Fig. 26.4 5 ON 3 SINTERED GLASS FILTER

### CLEANING

This is comparatively easy. After use the filters are immediately soaked in water and then the following are drawn through:

- 1 Water, to remove as much medicament as possible.
- 2 Hot (i.e. at about  $80^\circ\text{C}$ ) concentrated sulphuric acid containing 1 to 2 per cent of sodium nitrate, to oxidise deposits (Chromic acid cleaning solution is not suitable see 'Unglazed Porcelain').
- 3 Water, until the medium is free from acid. Final flushing is done with apyrogenic water.

### D GRADOCOL (COLLODION) MEMBRANES

In 1931, Elford developed techniques for preparing thin mucroporous plastic membranes based on cellulose nitrate. These were accurately reproducible, unlike similar methods used previously. The porosity of the membranes could be varied by altering the amounts and nature of the ingredients (which were colloidion and various solvents), and media, with very uniform pores, covering a range from  $3\text{ }\mu$  down to  $10\text{ }\mu$ , were produced.

For many years these filters were marketed in this country as Gradocol membranes but now they are almost unobtainable. Grades with a porosity of about  $0.5\text{ }\mu$  were used for bacterial filtration. They consist of thin discs 3 to 4 cm in diameter and are supported for use on a perforated plate in a special metal holder, this is of heavy construction because high pressures are needed for the ultra fine grades used for virus filtration.

#### *Advantages*

- 1 Bacteria are removed mechanically by sieving, because the pores are so fine. Therefore, there is little danger of organisms gradually passing through the pores to contaminate the filtrate, this may occur with ceramic, asbestos-cellulose and glass types if filtration is prolonged.
- 2 Because the membranes are paper thin, adsorption of medicaments is usually insignificant.
- 3 The flow rate is high because most of the membrane consists of pores, there is far less inter pore matrix than in fibrous or sintered filters.
- 4 A new disc is used for each filtration.

#### *Disadvantages*

- 1 They must be stored in sterile apyrogenic water because they become distorted and useless if allowed to dry.

2 They are readily attacked by certain fungi (Moulds are well endowed with cellulose destroying enzymes) This is prevented by re sterilisation of their container (usually a flat jar with a metal screw cap and rubber liner) after each time of opening Alternatively a harmless fungistat, e.g. an ester of parahydroxybenzoic acid, may be added, but then the disc must be well washed with sterile water before use

3 They cannot be sterilised by autoclaving Steaming or boiling in water for an hour were recommended but as these methods cannot be relied upon to kill spores, Gradocol membranes are not used for sterilising parenteral solutions

4 They are expensive

### E MEMBRANE FILTERS

These were developed more recently They consist of pure cellulose acetate (Oxoid Membrane filters, manufactured by Oxo Ltd., Thames House E C 4), mixtures of cellulose esters (Millipore filters, manufactured by the Millipore Filter Corporation, Bedford, Mass., U.S.A.) or mixtures of cellulose esters and regenerated cellulose (Membranfiltern, manufactured by Sartorius-Werke A G and Co., Göttingen, Germany)

Oxoid membranes (Oxoid Manual, 1961) are made in diameters from 1.7 to 14 cm, but the 5 and 6 cm sizes are most popular The 1.7 and 2 cm sizes are used in centrifuge and syringe-type holders respectively (see 'Filtration Units') The discs are about  $120\ \mu$  thick and the pores range from 0.5 to  $1\ \mu$  (mean 0.6) on the upper surface and gradually widen to from 3 to  $5\ \mu$  on the lower surface

Millipore filters are available in 12 grades covering pore sizes from  $8\ \mu$  to  $10\ \text{m}\mu$ . The HA grade which has a pore size of  $0.45 \pm 0.02\ \mu$  is generally used for bacterial filtration, but an alternative grade, GS, of pore size  $0.22 \pm 0.02\ \mu$  is recommended for materials such as blood products in which very small bacterial contaminants (e.g. certain pseudomonads) are occasionally found.

The German membranes are produced in several grades, ranging from 1 to  $0.1\ \mu$  in pore size Ultra-filters, that must be kept moist (possibly they contain a large proportion of cellulose nitrate) are also available, in porosities from 100 to  $5\ \text{m}\mu$

#### *Advantages*

1 to 4 As for Gradocol membranes

Examples of the high flow rates obtainable are—

(a) Oxoid membranes At  $20^\circ\text{C}$ , 500 ml of water pass through a 5-cm membrane in 5 min when the pressure differential between the two surfaces is

equivalent to 250 mm of mercury, which is generally sufficient in practice but can be increased if the flow rate slows, due to blockage, during filtration.

(b) Millipore filters HA. At  $20^\circ\text{C}$ , 65 ml of water pass through each  $\text{cm}^2/\text{min}$  when the pressure differential is 700 mm (The comparable figure for the Oxoid membrane, which has larger pores, is 80 ml.) The G S grade passes about  $\frac{1}{2}$  of this volume under the same conditions

With fibrous pads, the fastest of the more conventional media, the flow rate is only about 6 per cent of the above This difference is largely because in membrane filters the pores occupy about 80 per cent of the total filter volume while in ceramics and fibrous pads the comparable figure is only about 10 per cent

5 All except German ultrafilters can be stored dry In this condition they should be handled carefully, e.g. with flat tipped forceps, because they are rather brittle They become very tough when wet

6 Most types, including the Oxoid membranes and Millipore HA and GS filters can be sterilised by autoclaving For the former 15 min at  $121^\circ\text{C}$  is recommended Preferably each membrane should be sterilised in its holder but if numbers of them are sterilised separately they must be supported by interleaves of thick absorbent filter pads, then totally wrapped in paper and placed in a flat tin that is perforated to admit steam. This procedure prevents curling The German filters can be obtained ready-sterilised (by ethylene oxide gas) and packed in bacteria proof polythene envelopes Some grades of Millipore membranes are packed in an inner envelope of polythene and an outer of a polyester film and sterilised by high energy electrons (see chapter 28)

7 They do not yield particles or chemical substances to the filtrate

8 They can be used for sterility testing by filtration

9 Organisms collected from liquids or gases (see 'Air Sampling') can be counted and studied by aseptically transferring the membrane to a thick pad of absorbent filter paper (e.g. Whatman 17) previously saturated with a nutrient medium and incubating in a round tin or plastic box (Fig. 26.5)

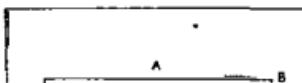


Fig. 26.5 INCUBATION TIN FOR MEMBRANE FILTERS

A—membrane filter  
B—medium-saturated absorbent pad

Because the membranes are so thin and porous the medium readily diffuses up the pores to the organisms which multiply to produce visible colonies on the surface To facilitate counting, the upper surface is usually marked with a grid Several precautions are necessary when using this technique—

(a) The special membrane media should be used for best results (Oxoid Manual, 1961)

(b) The pad must not be flooded with medium A suitable volume for the 5 cm size is 2.5 ml If liquid overlaps the membrane edge it spreads the colonies Pads already impregnated with nutrients and to which only sterile water need be added are available for the German filters

(c) The membranes must be in contact with the pads at all points because air locks prevent medium from reaching the organisms above One edge of the membrane should be brought into contact with the pad, and the rest allowed to roll gently on to the surface

(d) The lid of the container must fit well to prevent excessive evaporation of the small volume of medium during incubation The lid is kept uppermost

#### *Disadvantages*

Compared with the older types of media—

1 Membranes clog more easily because dirt and organisms are almost entirely retained on the upper surface Fibrous pads are greatly superior in this respect The Millipore Filter Corporation makes thin glass fibre paper prefilters to overcome this problem Pharmaceutical solutions contain few particles and rarely cause difficulty

2 Their chemical resistance is less They are soluble in certain organic solvents, e.g. ketones and esters Occasionally this is an advantage, e.g. the membrane can be dissolved away when separation of the retained organisms is desired

(For further information on membrane filters, see Windle Taylor, Burman and Oliver, 1953 and Millipore Corporation, 1961, 1963)

### FILTRATION UNITS

Bacteria proof filters are used in units designed to facilitate aseptic collection of the filtrate and, usually and desirably, aseptic distribution into the final sterile containers Their basic design resembles an aseptic distribution unit (q.v.) but the tube through which liquid is admitted in the latter is either connected to or replaced by the filter Modifications are legion and a list of references to many will be found in a paper by Elliott and Rutter (1952)

The essential features are the same as for distribution units and, again, the most important is a minimum number of joints because each is a potential contamination point

Pressure on or vacuum below the liquid is necessary Generally, pressure is preferred and the B.P.C. recommends it The advantages are

- 1 Unsterile air cannot enter leaking joints With vacuum it is sucked in
- 2 Filling can be done while the liquid is filtering
- 3 Evaporation and foaming of the filtrate is reduced The latter can be a problem with protein containing preparations and culture media

Nevertheless, there are points in favour of vacuum

- 1 It tightens loose joints
- 2 The closed system, essential for pressure filtration, is unnecessary

In fact, either method can be safely used for simple aqueous solutions if the unit is assembled efficiently

Pressure can be applied with a hand pump (a robust version of a bicycle pump but with an internal valve), a cylinder of air or nitrogen fitted with a good reducing valve, or an electrically operated oil sealed pump The latter is the best source of vacuum A reservoir is desirable between the unit and the supply point to protect the filtration medium and the joints of the unit from sudden sharp changes of pressure

#### 1 Units for Sintered Ceramics

##### (a) VACUUM TYPES

Examples are given in Fig. 26.6 The type of filter shown in Fig. 26.6(2) should not be used when contact with metals must be avoided

##### (b) PRESSURE TYPES

As Fig. 26.7 suggests, it is less easy to assemble filter candles into pressure units

Batteries of large candles are occasionally used industrially At the other end of the scale, very tiny units with candles only about  $\frac{1}{2}$  in long have been invented for filtering and filling the liquid for one or two Chubritic vaccine bottles (e.g. see Lax, 1938)

#### 2. Units for Fibrous Pads

##### (a) VACUUM TYPES

These are Buchner funnel shaped and made from phosphor-bronze or nickel silver alloy, in both cases silver plated They are available for 3.6 and 6 cm pads There are several designs (Fig. 26.8) all of which can be separated into two parts either by

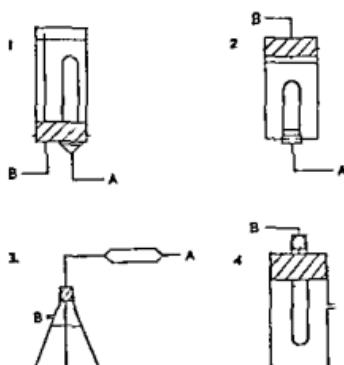
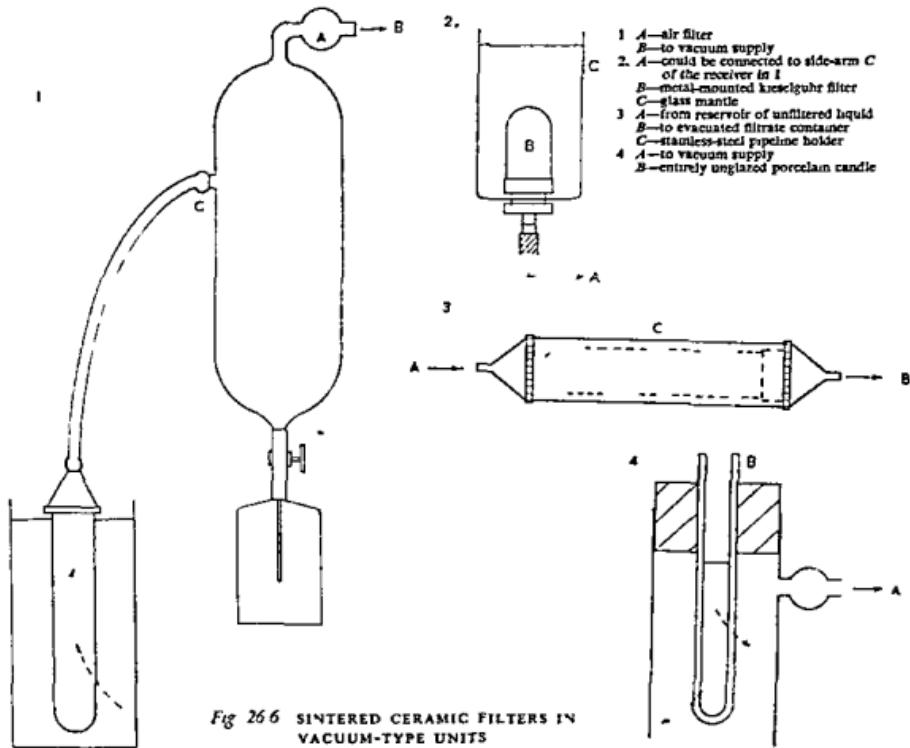


Fig. 26.7 SINTERED CERAMIC FILTERS IN PRESSURE-TYPE UNITS NUMBERS INDICATE CORRESPONDING VACUUM UNIT IN FIG. 26.6

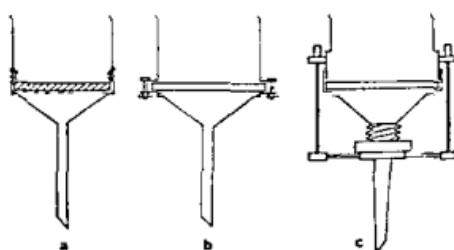


Fig. 26.8 VACUUM-TYPE HOLDERS FOR FIBROUS PADS

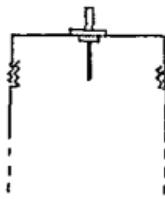


Fig. 269 TOP OF PRESSURE TYPE HOLDER FOR A FIBROUS PAD

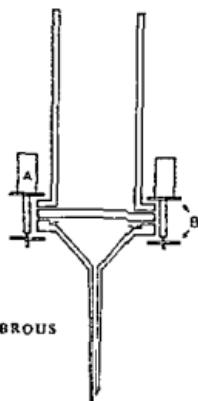


Fig. 2610 GLASS HOLDER FOR FIBROUS PAD FILTER  
A—screws  
B—metal clamping rings

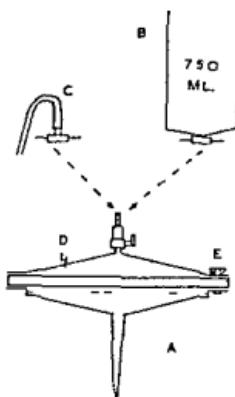


Fig. 2611 HOLDER FOR LARGE FIBROUS PAD  
A—outlet  
B—funnel  
C—connection and tubing to reservoir  
D—air bleed  
E—one of a ring of wing nuts

loosening wing nuts or unscrewing. The lower portion contains a wire grid or a perforated plate on which the pad is supported. The type shown in Fig. 268b has the disadvantage that the edge of the pad is exposed, it would be unsuitable for filtration sterility testing (*q.v.*)

#### (b) PRESSURE TYPES

The only important difference from the vacuum types is the lid (Fig. 269) which, in modern holders screws on. It carries a connexion for the tubing from the pressure source (*See also Wokes, 1936*)

#### (c) GLASS TYPE

This is a vacuum unit for substances requiring protection from metallic contact (Fig. 2610). In time, the plating of metal holders wears thin and may result in contamination of filtering liquids with harmful heavy metal ions. In the glass type the only metal part that the solution touches is the perforated supporting disc, which is made from stainless steel and is highly polished to resist corrosion.

All the above holders can be easily assembled into simple units similar to the one shown in Fig. 263

#### (d) LARGE HOLDERS FOR SINGLE PADS

These are used with 14-cm pads and are of gun metal heavily silvered (Fig. 2611). The inlet can be fitted with a special funnel of 750 ml capacity, for vacuum filtration, or connected via tubing to a reservoir to which pressure can be applied. The outlet is normally connected to a large receiver from which the filtrate can be fed to a filling unit (e.g. a burette with a two-way tap) either during (if pressure is used) or

after filtration. A layer of nylon mesh or a disc of sintered stainless steel can be placed beneath the pad to collect fibres. Alternatively, a clarifying sintered-glass filter can be included in the line to the receiver.

#### (e) FILTER PRESSES

Large square pads (e.g. 20 cm by 20 cm) are used in the normal horizontal filter presses. In addition, British Filters Ltd., of Maidenhead supply a vertical glass press for 14 cm pads, this is particularly suitable for filtering large volumes of parenteral solutions. It will withstand steam sterilisation because the glass is borosilicate and although some parts are metal, liquid touches only the glass and the pads.

#### (f) SYRINGE UNITS

In the United States a syringe unit similar to the one available for membrane filters in the U.K. is used for the sterilisation of very small volumes.

#### (g) CENTRIFUGE UNITS

These are usually known as Hemmings filters and are manufactured by Beaumaris Instrument Co., Beaumaris. The unit (*A* in Fig. 2612) is a small metal collar threaded at both ends to connect two bijou (5 ml) bottles *D<sub>1</sub>*, *D<sub>2</sub>*, one of these contains the liquid to be filtered and the other acts as a receiver. In the centre is a perforated diaphragm



Fig. 26.12 HEMINGS FILTER

which supports a 17 cm Grade SB Sterimat, C, on one side while from the other extends a short delivery tube around which is fitted a rubber sealing ring, B. The receiver screws against this ring while the bottle of unsterile liquid is sealed by the filter pad.

The complete assembly is placed, empty bottle at the bottom, in a 50-ml centrifuge bucket and is balanced against a duplicate unit containing an equal volume of liquid. If larger buckets are available 10-ml bottles can be used instead of the bijou type because both have the same neck size. Units for Universal containers (30-ml wide-mouthed McCartney bottles) are available.

The assemblies are centrifuged at about 2,500 rev/min (equivalent to about 14 lb/in<sup>2</sup>) and at this speed 2 ml of serum is filtered in 3 to 4 minutes. A maximum of 0.4 ml is lost through absorption in the pad. Wyllie (1955) recommends that some of the liquid should not be filtered, because if none is left after centrifugation the filtrate may be contaminated since the pad is less efficient if it is not completely saturated.

The unit has been recommended as an easily assembled, sterilised and cleaned apparatus for the filtration of small quantities of urgently needed injections and ophthalmic solutions but because of the risk of contamination with loose fibres it is much less satisfactory for these purposes than the more recent, modified unit for microporous membranes (q.v.).

### 3. Units for Sintered-glass Filters

Filters in which the medium is fused into Buchner funnel type holders are the most popular. They can be fitted into simple units such as the one illustrated in Fig. 26.6(4). Alternatively, the stem may be fused to a suitable receiver to eliminate a rubber connexion (Sykes, 1958a).

A self-contained filtration unit with interchangeable sintered glass and fibrous pad holders was invented by Barfield (1955), Fig. 26.13. The only rubber component is the tube to the vacuum supply, the rest have been replaced by ground glass joints which apart from overcoming the many disadvantages of rubber, give the unit much greater rigidity. This is enhanced by housing the unit in a metal box which, in use, is attached at the back to a retort stand. The

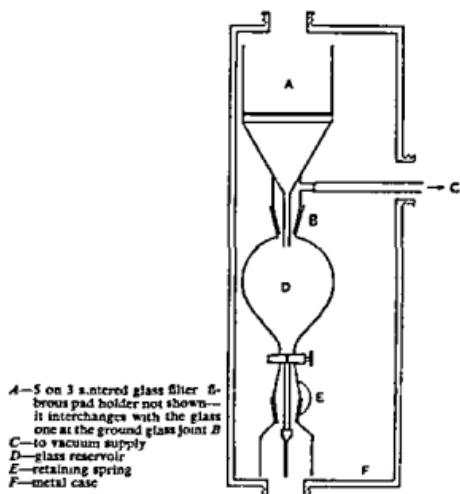


Fig. 26.13 THE BARFIELD UNIT

box is lidded and has screw caps to the three openings. All joints are lightly lubricated with silicone grease. Sterilisation is by dry or moist heat but for the latter the caps must be replaced by steam permeable closures, e.g. paper caps or muslin-covered plugs. The high degree of enclosure of the unit during filtration, within a case that is also sterile internally, is a particularly valuable feature. It was available commercially for a time and although manufacture has been discontinued it remains an outstanding example of thoughtful design.

### 4 Units for Membrane Filters

Oxoid membranes can be used in the following holders

#### (a) METAL FUNNELS

The best known is the Gallenkamp type (Fig. 26.14) which takes 5-cm membranes. The base A carries a sintered carbon or a Grade-1 sintered glass disc B which fits inside a silicone rubber gasket C and supports the membrane D. Another gasket E is placed on top of the membrane to protect it from the flanged bottom of the cylindrical funnel top F when this is clamped tightly in position by the twist action sealing ring G. Apart from the funnel, which is anodised aluminium, all parts are heavily nickel-plated. The standard funnel top holds 100 ml but can be replaced by a 500-ml size if required. For use

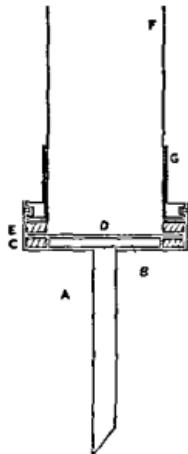


Fig 26 14 GALLENKAMP HOLDER FOR MEMBRANE FILTER

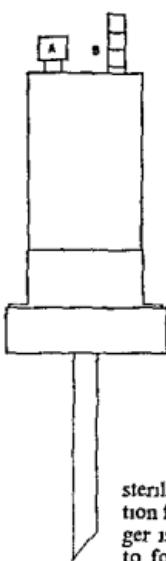


Fig 26 15 PRESSURE UNIT (GALLENKAMP) FOR MEMBRANE FILTER (EXTERNAL VIEW)

with pressure a closed vessel (Fig 26 15) is available instead of the open funnel, it has a nozzle *B*, for the pump connexion, and a pressure release valve *A*

#### (b) GLASS FUNNELS

Sizes for 5 and 6 cm membranes are obtainable They are similar to the all glass holders for fibrous pads but the two parts are held together by external springs and the membrane is not exposed at the edge As the support is a sintered glass disc there is no contact with metals

#### (c) CENTRIFUGE UNIT

A Hemmings filter (see Fibrous Pad Holders), modified for use with 1 7-cm membranes, is available

#### (d) SYRINGE UNIT

This is a glass and metal syringe with a threaded connexion for the attachment of a small filter unit at the lower end of the barrel (Fig 26 16) The unit contains a perforated metal disc to support a strengthened 2-cm membrane, a rubber gasket is placed on the latter, and the whole is loosely screwed on to the syringe The plunger and barrel are sterilised apart (see chapter 29) and each part can be wrapped in paper or packed in a metal-capped tube After

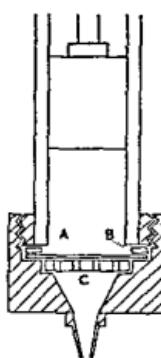


Fig 26 16 SYRINGE UNIT FOR MEMBRANE FILTER

*A*—membrane *B*—gasket  
*C*—perforated disc

sterilisation the unit is screwed up tightly, the solution for filtration is poured into the barrel, the plunger is inserted, and firm steady pressure is applied to force the liquid through the membrane into a sterile container The needle may be fitted before or after sterilisation (see chapter 29) Suggested uses are the sterilisation of small volumes of eye-drops and injections in emergencies For further information, see Smith and Mitchell (1962) and the technical literature of R B Turner & Co, Church Lane, N 2

A variety of cleverly designed and beautifully made units are available for Millipore filters

#### Aspects of the Sterilisation of Units

1 Check that the cannulae contain stilettos, these ensure that the bore is, and remains, clear

2 Plug or cover the openings of dust hoods and receivers and the tops of filter holders Although the latter do not come into contact with the filtrate this protection helps to minimise the number of organisms and particles to be filtered and, therefore, delays clogging

3 If the receiver has a rubber bung it is better to sterilise the latter and its fitments separately, this prevents adhesion of the rubber to the glass and keeps the unit short Long units are harder to pack and are difficult to fit into a small autoclave The bung must be inserted tightly when the two parts are assembled immediately before use

4 Autoclaving is the usual sterilisation method, and is essential if the unit has rubber or pvc parts Dry heat is preferable for sintered glass filters but they should be heated and cooled slowly to prevent

internal strain, which may crack and loosen the fine porosity layer

5 When a membrane filter is assembled in its unit before sterilisation the following precautions are advisable to prevent distortion

(a) The filter should be kept flat. Lightbown (1962) used a perforated stainless steel disc large enough to cover the whole of the effective filtration area. This could be removed, before filtration, by means of threaded handle

(b) The holder should not be fully tightened until after sterilisation, a precaution that is also desirable with fibrous pad filters

#### 6 Heat sealed bags are convenient containers

### APPLICATIONS OF THE VARIOUS MEDIA

In the U.K., the media most widely used for the sterilisation of parenteral solutions are sintered glass and fibrous pads. Sintered filters are popular for small volume injections in hospitals because they are easily assembled or fused into units, yield virtually nothing to the filtrate and are not difficult to clean. Asbestos pads are often preferred in industry (e.g. for the injections prepared from animal products) because of their suitability for large volumes, particularly when assembled in filter presses.

Ceramics have been most widely used in the immunological field.

Some bacteria are smaller than the widest pores of Oxoid membranes and, since retention is largely by sieving, it is possible (at least theoretically) for contamination of the filtrate to take place. Consequently, these filters have not been widely used for the sterilisation of parenteral solutions. However, Sutaria and Williams (1962) have successfully sterilised eye drops with them. Sintered glass supporting discs were used and a separate one was kept for each solution, to avoid chemical cross-contamination.

In America, sterilisation by filtration through fine-pored Millipore filters is well-established. For large-scale purposes, units similar to the 14-cm types described for fibrous pads are available. These have legs for bench mounting and are of stainless steel and have P.T.F.E. sealing surfaces to prevent sticking during sterilisation. Two sizes, for 14.2 and 29.3-cm membranes are produced. The initial flow rates of intravenous solutions through the HA grade at 15 lb/in<sup>2</sup> differential are 7.5 and 25 litres/min for the 14.2 and 29.3 cm membranes respectively. Up to 100 (14.2 cm) and 500 (29.3 cm) litres can be passed before clogging occurs. The HA grade is recommended for intravenous fluids, ophthalmic solutions

Water for Injection and small volume injections, and the GS grade for insulin and blood products

### FILTRATION OF OILS

Filtration is a practicable alternative to dry heat for the sterilisation of oils. Sykes and Royce (1950) have reported the successful use of dry Sterimats, Grade SB, for hundreds of batches of oily solutions. Doulton and Berkfeld candles of bubble pressure ( $q/v$ ) 18 lb/in<sup>2</sup> were also found to give sterile filtrates but were too slow to be of practical value on a large scale. The Millipore Corporation recommend the HA membrane and suggest that the oil should be prewarmed, to increase the flow rate, and preclarified to minimise clogging.

(See also Avis and Gershenson, 1955.)

### FILTRATION AS AN ALTERNATIVE TO A HEATING PROCESS

Although the B.P. often allows filtration as an alternative to a heating method it should not be chosen indiscriminately. Manufacturing houses often prefer it because the actual sterilisation process is quick, and the slight deleterious changes that take place in some normally heat sterilised injections are avoided. In addition, simplification of the formula may be possible if the hazard of heat exposure is eliminated. However, manufacturing houses usually have superlative facilities for the maintenance of asepsis during the process and for the performance of the subsequent sterility tests. Equally good conditions are rare in other spheres of pharmaceutical practice and therefore, the risk of bacteriological breakdown of the process or tests is much greater, in these circumstances filtration should be used only when there is no alternative, and certainly not to replace the more reliable heating methods.

### THE TESTING OF FILTERS

This involves tests—

1 To show that the medium retains organisms satisfactorily. For non-disposable filters this type of test must be done before a new filter is used and at intervals during its life. For disposable types reliance is placed on the routine tests performed by the manufacturer on samples from each production batch.

Proof of efficiency is obtainable in two ways:

- (a) Directly, by attempting to filter a suspension of a very small organism (Bacteriological techniques)
- (b) Indirectly, by finding the maximum pore size and assuming that if this is no greater than the maximum in previously satisfactory filters of the same type the filter will be bacteria proof (Bubble pressure techniques)

2 To show that the flow rate of aqueous fluids is reasonably fast A filter with a satisfactory maximum pore size might have a high percentage of much smaller pores and therefore filter extremely slowly

### 1a Bacteriological Techniques

These are the most convincing efficiency tests An example is described in the *British Pharmaceutical Codex*

A suitable bacterial suspension is made by diluting 4 ml of a 48 hr broth culture of a chromogenic (pigment producing) strain of *Serratia marcescens* (formerly known as *Chromobacterium prodigiosum*) to 100 ml with nutrient broth This organism is chosen because it is small (0.3 to 1.3  $\mu$  long and 0.3 to 0.4  $\mu$  wide) and aerobic, it grows vigorously and, if incubated at 25°C, produces a bright red pigment that aids its detection

The filter is assembled into a unit which is then sterilised Afterwards the unit is set up aseptically and the suspension is filtered at a pressure differential of not less than 400 mm Since differentials lower than this are normally used (e.g. 150 mm), at least at the start of filtration, if the filter is impermeable at the test pressure it should be satisfactory under conditions of use

Fifty ml are collected aseptically, preferably the first 50 ml because the medium will gradually block as filtration progresses and pores that at the beginning will pass bacteria may not do so later

The container is aseptically closed and incubated at 25°C for 5 days There must be no growth of *Serratia marcescens* or any other organism

Sykes (1958b) has suggested improvements to this test The following are particularly interesting

1 Reduction of the incubation time of the initial culture to 24 hr—because the organism ages easily and aged strains are larger and, therefore, less able to penetrate a filter

2 Increased dilution of the culture (200 instead of 25 times) and collection of a smaller volume (15 ml instead of 50 ml), because the prescribed suspension is rather concentrated and quickly clogs filters of small surface area

3 An increased pressure differential (700 mm instead of 400 mm), because, in practice, modern pumps if not very carefully used may subject filters to pressures as great as this

4 Rejection of the filter only if *Serratia marcescens* appears in the filtrate This is because other organisms have almost certainly come from a leaky unit or bad technique and not from a faulty filter Sykes recommends that containers in which growth

of *Serratia marcescens* is uncertain should be subcultured on slopes because pigment production is especially good on solid media Then if the test organism is absent the filter should be sterilised, cleaned and retested with particular care to prevent recurrence of accidental contamination

5 A test to confirm that the filter is not yielding inhibitory substances, e.g. residual cleaning agents Filtrates that are sterile after incubation should be lightly inoculated with the test organism and re-incubated for 24 hr

The Millipore Filter Corporation applies efficiency tests to samples of the material used for its membranes For example, the test for the HA grade involves passing a suspension of *Serratia marcescens* containing  $5 \times 10^9$  organisms per ml, first through the test and then through a control membrane The latter is incubated on a medium soaked pad in the usual way and examined for colonies of the test organism The material fails if any colonies are found A test for absence of inhibitory substances, which is particularly important for these membranes because often bacteria are required to grow on them (see p 484) is performed on the HA filters by comparing the number of colonies and amount of pigment produced on the membranes and on agar plates by equal volumes of a suspension of *Serratia marcescens*

### 1b Bubble-Pressure Techniques

These methods are applicable to rigid media only, i.e. sintered ceramics and glass

Maximum pore diameters cannot be measured directly because of irregularities within the pores but they can be calculated from the pressure necessary to force the first bubble of air through the medium when it is wetted with a liquid of known surface tension, because the first bubble forms at the pore of greatest diameter

The filter is cleaned, sterilised, dried and soaked in the test liquid Water is used for clarifying filters but very high pressures are required to blow air through bacteria proof types against water and, therefore, a liquid of lower surface tension is more satisfactory Carbon tetrachloride (surface tension—27 dynes/cm) is suitable (Compare the surface tension of water—approximately 73 dynes/cm) The soaking expels the air from the pores It can be done by placing the filter in a suitable vessel, evacuating to at least 700 mm Hg and, while holding the vacuum, admitting the liquid until the filter is covered (Alternative methods are suggested in B S 1752 1963) Afterwards the filter is connected to a compressed air system that can be controlled accurately, e.g. an

A—compressed air  
B—fine adjustment valve  
C—shut-off valve  
D—pressure gauge  
E—disc filter containing layer of test liquid  
F—candle filter immersed in test liquid

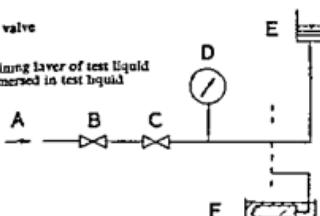


Fig. 26.17 APPARATUS FOR PORE SIZE DETERMINATIONS

oil sealed pump linked through shut-off and needle valves. Candles are immersed in a shallow trough of the test liquid but for disc filters it is usually more convenient to pour a thin layer on the upper surface (Fig 26.17).

Pressure is applied very gradually via the needle valve. If the filter is cracked or badly sealed to its holder large bubbles appear at low pressures but normally the first bubble rises from the largest pore. The pressure at which this occurs is noted and the maximum pore size calculated from the formula—

$$D = \frac{30\gamma}{P}$$

where  $D$  = the pore size in microns,

$\gamma$  = the surface tension of the liquid in dynes/cm,

$P$  = the pressure in mm of mercury.

With carbon tetrachloride a pressure of 400 mm Hg is approximately equivalent to a pore size of  $2\text{ }\mu$ , this is given as the maximum pore size of a grade 5 filter in B S 1752 1963.

The porosity of certain bacteria proof filters (e.g. the American Mandler) is expressed by the pressure required to force air through against water (the bubble pressure). For example, rigid filters with bubble pressures of 21 and 16.8 lb/in.<sup>2</sup> have maximum pore sizes of 2 and  $2.5\text{ }\mu$  respectively. Royce and Sykes (1950), using unglazed porcelain and kieselguhr media, found that filters with bubble pressures of 17 lb/in.<sup>2</sup> or more, gave sterile filtrates for several hours.

#### Uniformity of Pore Diameter

This determination shows that most of the pores are not very much smaller than the maximum and that the pore distribution is even over the whole surface of the pad.

After the first bubble has broken away, in the determination of maximum pore size, the pressure is increased by a specified amount (e.g. by 150 mm

for a grade-5 filter with carbon tetrachloride) and bubbles should escape evenly from all parts of the filter surface.

#### 2. Permeability to Water

It is difficult to obtain reproducible results for the rate of flow of water through a bacteria-proof filter. Therefore, the permeability to air is determined in a special apparatus (B S 1752 1963) by measuring the rate of flow of air through a defined area of the filter with a known pressure difference between the faces. The result is expressed as millilitres of air, per minute, per square centimetre of filter, per 10 mm water pressure difference. A satisfactory value for a grade-5 filter is 0.1 ml/min/cm<sup>2</sup>/10 mm H<sub>2</sub>O. This is approximately equivalent to a water flow rate of 4.5 ml/min for a disc of 30 mm effective diameter under a vacuum of 250 mm Hg.

#### ADVANTAGES OF BACTERIAL FILTRATION

1 The preparation is not heated. Therefore, the method is suitable for thermolabile medicaments such as immunological preparations, blood products, certain animal products (e.g. insulin) and enzymes (e.g. hyaluronidase).

2 Both dead and living organisms are removed. In heating methods the killed organisms remain in the preparation.

3 If the solution has been prepared carefully, to reduce foreign particles to a minimum, and the volume is small, clarification and filtration can be carried out at the same time.

4 If sterile filters, units and containers are always kept available, filtration is an excellent method for the rapid supply of a small volume of a parenteral solution in an emergency.

5 Eye drops are usually heat sterilised but it is difficult to obtain a dropper bottle that, when filled and closed, will withstand the sterilisation conditions. Filtration (e.g. through a membrane filter) overcomes this problem because the container is sterilised separately and in two parts (bottle and dropper top).

#### DISADVANTAGES OF BACTERIAL FILTRATION

1 Aseptic technique is involved and necessitates

- (a) Highly trained staff who are fully aware of the limitations of the method and
- (b) Sterile equipment and facilities for its preparation.

2 It cannot be the last stage of preparation because it is only applicable to bulk liquids. During the distribution of the filtrate into, and the closure of,

the final containers there is risk of accidental contamination. Therefore, a bactericide is advisable in all filtered injections of *small volume*, both single and multi-dose.

3 Sterility tests, with their associated problems of design and interpretation, are required on samples from every batch. Except in an emergency, issue is not permitted until the tests have been passed.

4 Although the number of viruses present is reduced by adsorption, viral sterility cannot be guaranteed. However, because of the absence of living cells, essential for their growth, viruses cannot multiply in pharmaceutical solutions. A few bacteria produce forms that pass all but the finest filters.

5 Faults in the media are not immediately detectable. Non-disposable filters must be regularly checked by bacteriological efficiency tests (preferably) or by pore-size determinations.

6 Units may leak if carelessly assembled. It is advantageous to have few joints and to use pressure rather than vacuum. Lining of joints with sterile non-absorbent cotton wool is another possible precaution. The joint between the disc and holder in the type of fibrous pad unit in which the edge of the disc is exposed (Fig. 26.8b) is an example of a potential hazard.

7 Adsorption of medicaments may occur. This is most significant with kieselguhr candles and, to a lesser extent, fibrous pads. It is least important with membranes. Filtration should not be used for small volumes of solutions of medicaments that are seriously adsorbed. With large volumes the problem can be overcome by rejecting the first part of the filtrate.

8 If filtration is prolonged, organisms may pass through the media that do not retain by sieving. For example, with sintered glass filters contamination of nutrient solutions has occurred after 18 hr. The organisms involved are small and usually motile, e.g. vibrios and spirochaetes. The problem is simply overcome by avoiding long filtrations.

9 Most of the media have disadvantages, e.g.

Unglazed porcelain	—cleaning —retention of a considerable volume of the filtrate in the medium. —adsorption
Kieselguhr	—adsorption
Fibrous pads	—yield particles and alkali —adsorption
Glass	—weak when large
Collodion (Gradocol)	—storage and sterilisation are difficult
Membranes	—clogging

10 Filtration is unsuitable for suspensions.

11 It can only be used if the medicament is stable in the solvent at normal storage temperatures.

## MECHANISMS OF BACTERIAL FILTRATION

### 1. Mechanical Sieving

Fine porosity filters, such as microporous plastics, retain organisms because their pores are smaller than bacteria. If this sieving process was the only mechanism, sintered media and fibrous pads, in which the majority of pores are larger than bacteria, would not be bacteria proof.

### 2. Retention on Pore Irregularities

An organism passing along a pore in a sintered or fibrous filter travels a tortuous path that has a very uneven surface. It has been estimated that there are about 2 000 irregularities/cm of pore in an unglazed porcelain candle. Bacteria may be stopped by or trapped in these hazards.

This theory suggests an explanation for the penetration of sintered and fibrous media after prolonged filtration. Bacteria, originally arrested on projections in the upper part of the filter become dislodged as filtration proceeds and, after a series of further temporary retentions, eventually appear in the filtrate.

### 3. Electrostatic Attraction

The efficiency with which particles can be retained by electrostatic attraction is well known to users of certain plastics (e.g. polythene) and synthetic fibres. It has been suggested that this force may assist the retention of bacteria within a filter.

Although attractive, this theory is difficult to substantiate. It would hold if bacteria were positively charged, because ceramic, glass and asbestos filters carry negative charges but, in fact, acidic groups predominate in all bacterial species (Dubos 1949). However, the overall charge on the organism may not be the important factor, and retention may take place through the basic groups of certain cell components, particularly proteins. The pH of the filtering solution would be expected to influence this mechanism by altering the basicity or acidity of the proteins.

Sykes and Royce (1950) have shown that Sterimats SB can be made permeable to bacteria by soaking in oil before filtering an aqueous suspension of organisms. It has been suggested that the oil insulates the charges on the filter. A similar effect was observed

with candle filters by Holman, but Sykes and Royce could not confirm this, probably because they used finer filters (bubble pressure 18 to 22 lb/in.<sup>2</sup>) in which mechanical filtration predominated.

Some surface active agents, e.g. certain soaps and bile salts, increase the permeability of filters, possibly by affecting the surface charge.

#### 4. Imbibition of Water

The cellulosic fibres in fibrous pads imbibe water from aqueous solutions. This causes the pad matrix to swell, with consequent reduction in the size of the interstices and more efficient retention of bacteria. Strongly alcoholic solutions cause less swelling and, therefore, organisms may pass through.

### PREPARATION OF AN INJECTION STERILISED BY FILTRATION

*Sterilisation Method*—Bacterial filtration

*Type of Injection*—Single dose of small volume and multi-dose

#### EXERCISE 261

*Six 1-ml ampoules and two 10-ml multidose injections of Aneurine Hydrochloride Injection B.P.*

- 1 The B.P. states that when no dose is prescribed a solution of 25 mg in 1 ml shall be dispensed.
- 2 A bactericide will be included because
  - (i) Some of the containers are multidose.
  - (ii) The B.P. allows the addition of a bactericide to single-dose injections prepared by filtration, partly because there is no terminal sterilisation process and partly because post-sterilisation contamination might escape detection because of the limitations of sterility testing.
- 3 The container must comply with the test for alkalinity. Protection from light is necessary.

#### 4 Overage

This is 0.1 ml for each ampoule. Therefore,  $6 \times 1.1 + 2 \times 10 = 26.6$  ml is the minimum volume required.

Allowance must be made for

- (i) The solution absorbed by, or retained in the pores, of the filter medium.
- (ii) The extra containers necessary for the sterility tests. When containers of different types are filled from the same bulk volume samples of each should be tested. In this case one ampoule and one bottle will be needed—an extra 11 ml.

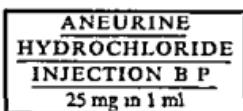
Therefore, 50 ml will be prepared.

#### 5 Formula

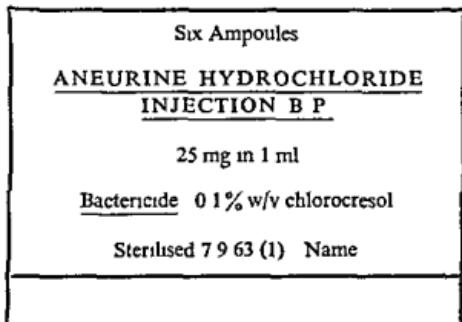
	B.P. amounts	Amounts used
Aneurine hydrochloride 0.1 per cent w/v chlorocresol in Water for Injection.	0.025 G	1.25 G
	to 1 ml	to 50 ml

#### 6 Labels

Aneurine hydrochloride is not a poison or a therapeutic substance  
*Ampoule*



Box

**PROTECT FROM LIGHT***Multi dose Containers*

As for ampoule box, but with '10 ml' instead of 'Six Ampoules', and '25 mg in 1 ml dose' instead of '25 mg in 1 ml'

*7 Preparation of the solution*

It is advantageous to make the solution aseptically, using sterile apparatus and solvent. Thus, by reducing the number of bacteria present, decreases the chance of failure of the process and extends the time before the filter clogs. It may be impracticable for large volumes but for a small number of injections it is strongly recommended, particularly if the working conditions and operator's experience are not superlative.

When this practice is adopted the dispensing procedure follows the principles outlined in chapter 24

- (a) The working area and the hands are prepared
- (b) The required amount of (sterile) aneurine hydrochloride is weighed in a sterile boat and transferred to part of the sterile solvent contained in a sterile flask covered with a sterile beaker. It is dissolved *without the use of heat*
- (c) The solution is transferred to a sterile graduated flask or stoppered cylinder, made up to volume and mixed
- (d) The balance and other unwanted articles are removed from the screen

*8 Filtration***STERILE APPARATUS AND MATERIALS REQUIRED**

*Filtration unit* In the U.K., at present, the usual filters for a volume of this size are sintered glass or asbestos. The former is preferable for reasons given previously. For details of units, packing and sterilisation methods the appropriate earlier sections should be consulted.

*(continued overleaf)*

*Exercise 26 I continued*

autoclaving is used the unit is dried afterwards to prevent dilution of the injection by moisture in the filter and bacterial contamination through damp plugs and air filters  
 Container of 10 ampoules, opened, annealed etc  
 Durham tubes  
 Three small Clinbritic bottles  
 Jar of rubber plugs or skirted caps equilibrated with and sterilised in chlorocresol solution  
 Sealing rings (if plug closures are to be used)

**METHOD****Prepare the containers**

Open, assemble and support the filter unit. Attach it to the vacuum source (Vacuum is chosen because a Buchner-type filter is most convenient for 50 ml and is not easy to use with pressure)

Tightly close the outlet from the receiver

Pour the solution into the funnel taking care not to let any run down the outside of the filter

Gently evacuate the receiver by opening the control on the tubing within the screen. It is an advantage if the external needle valve is preset to prevent an excessive vacuum. A pressure difference of 250 mm Hg should be adequate for the complete filtration of 50 ml

Afterwards, close the gate clip and carefully remove the vacuum tube from the unit so that air is admitted slowly. A more satisfactory arrangement is to have a T-connexion in the vacuum line, a gate clip on a rubber tube attached to the free arm of this can then be used to control the entry of air and prevent a sharp inrush

**9 Aseptic filling and sealing**

These must be carried out with scrupulous care because during these stages the solution is at most danger from accidental contamination

**10 Sterility testing****NEUTRALISATION**

The medicament can be ignored because it is a growth factor for many organisms

The effect of the chlorocresol can be removed by a 50 times dilution.

**SAMPLE SIZE**

The ampoule volume is less than 2 ml, therefore, 0.5 ml (i.e. half) quantities must be tested for aerobes and anaerobes

The multi-dose volume is more than 2 ml, therefore, 1 ml quantities must be used for the two tests

**MEDIA**

For the ampoule,  $25(50 \times 0.5)$  ml of aerobic and anaerobic media

For the bottle,  $50(50 \times 1)$  ml of each medium.

If a joint medium is used both aerobic and anaerobic samples are put into the same tube and, therefore, the volumes must be doubled i.e. 50 and 100 ml for the ampoule and bottle respectively

(continued)

**Exercise 26 I continued****CONTROLS**

Assuming that the batches of medium have been shown to be sterile and capable of supporting the growth of small inocula of exacting organisms, it is only necessary to confirm that the chlorocresol has been adequately neutralised, i.e. by setting up one tube each of aerobic and anaerobic medium, or two tubes of the joint medium, containing (i) the same amount of sample as in the test tubes and (ii) dilutions of appropriate test organisms.

When a batch containing ampoules only is being tested a second ampoule is required for the controls when the ampoule size is 2 ml or less, unless some excess (undistributed) solution is available. In the present case however, part of the residual solution in the sampled vaccine bottle can be used.

**TABLES**

*See Table 26 I*

**TECHNIQUES**

These involve the two types of aseptic transfer—ampoule to tube (or bottle) and vaccine bottle to tube (or bottle).

Remember to

- (i) Label the containers clearly  
Heat and cool cooked meat and, if necessary, thioglycollate media
- (ii) Inoculate anaerobic samples and organisms at the bottoms of the tubes
- (iv) Inoculate organisms outside the asepsis laboratory

**11 Issue of the products**

Label the containers immediately after preparation, and while the tests are in progress store them in a dust proof cupboard reserved for preparation awaiting the results of sterility tests. If they pass they can be packed, checked and issued in the normal way.

**Labelling of Therapeutic Substances**

Special requirements apply to substances controlled by the Therapeutic Substances Regulations. Most of these substances and their injections are sterilised by filtration and/or prepared aseptically.

*On the sealed container (i.e. the ampoule, cartridge, multi dose vial etc.)*

- 1 The name of the substance—in letters larger than the proprietary name, if present. The convention recommended in this book—of restricting capitals to the name of the substance or preparation—ensures compliance with this requirement.
- 2 The licence number (to manufacture or import)
- 3 The batch number
- 4 The potency

Note that 2 and 3 are additional to the information usually put on ampoule labels.

For an ampoule of Heparin Injection a typical label is

HEPARIN INJECTION B.P.	
25 000 units in 1 ml	
Batch No 10	Licence No 100
Manufactured 10 11 1964	

The date of manufacture is a special B.P. requirement for this injection.

*On the container or outer wrapper or package*

- 1 The name and address of the manufacturer of the final product
- 2 The date of completion of manufacture
- 3 The date up to which the potency is retained or the permitted toxicity is not exceeded.
- 4 The storage conditions
- 5 The nature and percentage of any added bactericide

Table 26 /  
Sterility Test on. 1 ml ampoule of Aneurine Hydrochloride Injection containing 0.1 per cent chlororesol as a bactericide

Label	Medicament	Medium		Neutralisation by	Organisms — 1 ml of a 1 in $1 \times 10^6$ dilution of an 18-24 hr culture of	Incuba- tion time	Ex- pected	Ob- served	Result
		Volume (ml)	Type						
1 When separate aerobic and anaerobic media are used									
Aerobic Test	TA	0.5 ml Injection	25	Nutrient broth	Dilution (Chlororesol)	5 days at 37°C	-	-	-
Anaerobic	TN	do	do	Cooked meat	do	do	-	-	-
Aerobic	CA	do	do	Nutrient broth	do	<i>Staphylococcus</i> <i>aureus</i>	do	+	-
Anaerobic	CN	do	do	Cooked meat	do	<i>Clostridium</i> <i>histolyticum</i>	do	+	-
2 When a joint medium is used									
Aerobic & Anaerobic Test	T A + N	0.5 ml of Injection at top and bottom	50	Thioglycollate	do		do	-	-
Aerobic	CA	do	do	do	do	<i>Staphylococcus</i> <i>aureus</i>	do	+	-
Anaerobic	CN	do	do	do	do	<i>Clostridium</i> <i>histolyticum</i>	do	+	-
Main Control									

Note that (i) separate controls are set up in method 2 although the test is joint.

(ii) rich control in method 2 contains the same amount of medicament as the test.

The tables for the multi dose container are the same except that twice the volume of medicament and media are used.

For ampoules, cartridges, tiny vials and other small containers it is usually more convenient to put these requirements on the larger labelling area of the wrapper or package. The latter is usually a box. For large vials and bottles they are often included on the container label.

An appropriate label for Heparin Injection is shown on the right.

Special information, such as the route for injection and the nature of the solvent is required for certain substances. The Therapeutic Substance Regulations must be consulted for full information.

The injections for which filtration is the only official method of sterilisation are listed in Appendix 4.

**Six Ampoules**  
**HEPARIN INJECTION B.P.**

25 000 units in 1 ml

Storage Not above 20°C

Bactericide 0.3% w/v Cresol

Date of Expiry 10.11.1967

Date of Manufacture 10.11.1964

Batch No 10 Licence No 100

Name and Address of Manufacturer

## II THE PACKAGING OF THERMOLABILE SOLIDS FOR INJECTION

Thermolabile injection medicaments that are not sufficiently stable in solution for sterilisation by filtration are supplied dry. They are dissolved in a sterile solvent when required for use.

If the solution is stable for a short time (e.g. a few days) when carefully stored (e.g. refrigerated) the solid may be packed in a small multi-dose container. In hospitals, solutions of this type of medicament are often made in the pharmaceutical department from bulk sterile solvent and are issued with detailed instructions on storage and shelf life.

If, however, the stability in solution is so low that immediate injection after preparation is necessary, single-dose containers are essential. Since this type of medicament often deteriorates in the solid state if allowed to become damp the best container is an ampoule, and special types with wide necks to facilitate filling are available (Fig. 21.11). They should be large enough to hold the required volume of solvent which should be supplied at the same time in liquid ampoules of appropriate size. It should not be assumed that the user will have a stock of the solvent available, particularly if it is not Water for Injection. Even with Water for Injection there is the danger that if an ampoule is not supplied the requirement may be obtained from a previously used and possibly contaminated multi-dose container.

Examples of the two classes of medicament are listed in the aseptic technique section of Appendix 4.

Sterility of these powders can be achieved by bacterial filtration immediately prior to aseptic precipitation or crystallisation and then either

- 1 Complete aseptic handling thereafter, or
- 2 Relaxation of asepsis during the succeeding stages (e.g. separation, drying, milling, sieving, granulation) and then terminal sterilisation using a gaseous disinfectant. This method will sterilise only the exterior of the particles but is sufficient because the precipitation or crystallisation is aseptically performed from a sterile solvent.

On a large scale, sterile powders are filled aseptically into ampoules and vials from sterile machinery. Two methods are used:

- 1 *Filling by weight* This requires elaborate and expensive machinery.

2 *Filling by volume* The volume corresponding to the required weight is found and the machines are set to deliver this into each container. The method is not very accurate for small volumes.

An alternative, that has been used for certain biological substances, (e.g. chorionic gonadotrophin) is to dissolve the material, distribute the volume containing the required weight into each ampoule and then remove the solvent by freeze-drying.

Small scale aseptic distribution will be illustrated by an example.

### EXERCISE 26.2

Prepare 6 ampoules each containing 0.2 G of phenobarbitone sodium for use in the preparation of phenobarbitone injection B.P.

- 1 Because this is a Schedule 4A poison the dose must be on the prescription and, therefore, the B.P. cannot give a dose to be dispensed if none is prescribed.

(continued overleaf)

*Exercise 26.2 continued***2 Overage**

None is prescribed for powder ampoules. This may seem unsatisfactory since the problems necessitating overage are the same as for liquid ampoules. However, as the following example shows, it is difficult to make a satisfactory recommendation.

Phenobarbitone Injection is prepared by dissolving 0.2 G in 2 ml of Water for Injection.

The overage for 2 ml is 0.15 ml and, therefore, 2.15 ml of solvent should be added to permit the injection of exactly 2 ml.

Consequently, the liquid ampoule must contain 2.15 ml plus appropriate overage, which for this volume is 0.3 ml, i.e. a total of 2.45 ml.

In addition, there must be sufficient excess medicament in the powder ampoule to give the correct strength when the corrected volume of solvent is added. In the example, the injection must contain 200 mg, in 2 ml and, therefore, an excess of 15 mg is required for 2.15 ml.

But even this does not allow for the inaccuracy due to the volume displacement caused by solution of the drug.

Combined with the foreseeable reaction of the user to the instruction to add 2.15 ml to the powder ampoule, these complications provide understandable reasons for ignoring overages for solids.

**3 Labels**

Solids for injection are labelled with the following information:

*On the Ampoule*

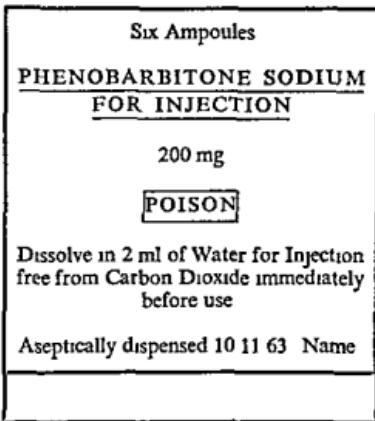
- 1 The name of the medicament followed by the words 'For Injection' or 'Sterile' in brackets. Following the convention used for liquid ampoules these should be in capitals and underlined.
- 2 The weight of medicament  
e.g.

<u>PHENOBARBITONE</u>
<u>SODIUM FOR INJECTION</u>
200 mg

*On the Box*

- 1 As above
- 2 The weight of the medicament in each ampoule
- 3 Directions for preparing the injection
- 4 The number of ampoules
- 5 The date of dispensing. The word 'Sterilised' would be inappropriate.
- 6 The batch number
- 7 Special instructions prescribed in the official monograph, e.g. the name and percentage of suspending, buffering and stabilising agents and the route for injection. Where relevant, the requirements of the Dangerous Drug Regulations, Poisons Rules and Therapeutic Substances Regulations must be complied with.

A suitable box label for the above ampoules, assuming supply other than on a prescription, would be



The word 'Poison' is in red because Phenobarbitone Sodium is a Schedule 1 poison

#### *4 Distribution*

Strict asepsis is essential

#### STERILE APPARATUS AND MATERIALS REQUIRED

Phenobarbitone sodium in a wide-mouthed screw-capped jar

Powder ampoules (e.g. the type shown in Fig. 21.10). Ten of the 2-ml size. These have open necks but should not be plugged because of the risk of fibres falling inside. Instead, they are covered with inverted ignition (or similar) tubes before sterilisation and packed upright in a tall covered beaker or tin.

Weighing boats with stems small enough to fit into the ampoule necks. (A suitable size is obtainable, to special order, from Britton Malcom Ltd.)

#### METHOD

Before scrubbing up, put a balance, test tube rack or ampoule block, and the jar of powder under the screen.

Afterwards—

Introduce a small sterile tile

Place the covered ampoules in the rack or block

Unwrap a boat and counterbalance it with sterile glass beads or with lead shot

Using a sterile spatula, carefully weigh 0.2 G of the medicament. (Note that the B.P. gives limits for the weights of powders in ampoules.) Have the weight checked.

Carry the boat to the first ampoule, preferably in flamed forceps. Remove the covering tube and stand it on its open end on the tile. At

(continued overleaf)

*Exercise 26 2 continued*

the same time insert the stem of the boat into the ampoule neck and, if necessary, gently tap the powder into the ampoule. Return the boat to the balance pan and at the same time replace the ampoule cover.

If suitable boats are not available Oxoid caps made into scoops (Fig. 24.25) can be used instead. Then a funnel is needed, and a suitable type is 1 in. in diameter with a stem  $\frac{3}{8}$  in. wide and 3 in. long. A protective cap for this can be made from an aluminium lid or a cut down 1½ in. Oxoid cap, in both cases a lip should be provided to facilitate handling with forceps (Fig. 26.18). The funnel can be sterilised in a capped tube and the cap in a screw-capped jar. Before the first weighing the cover of the first ampoule is replaced by the capped funnel. For filling, the cap is lifted with one pair of forceps while the weighing scoop is tipped with a second pair. Any powder that sticks in the funnel stem can be loosened



*Fig. 26.18 FUNNEL AND CAP FOR FILLING POWDER AMPOULES*

with a thick flamed and cooled wire, bent over at right angles at the top so that the hand does not move up and down immediately above the funnel mouth. After the transfer the cap is replaced, the scoop is returned to the balance pan, the capped funnel is interchanged with the cover of the second ampoule, and the weighing and filling are repeated. The cover on the tile is used for the last ampoule when the distribution is complete.

If, at any stage, contamination of the boat or scoop is suspected it is replaced with a fresh sterile one. Glass boats will not withstand flaming and even with metal scoops it is undesirable because if a few particles of powder have been left inside they may become charred and the residue may contaminate succeeding ampoules.

Eight ampoules should be filled to provide two for the sterility test Seal under a high screen. Leave the tube over the ampoule until it is on the sealing platform. The necks of powder ampoules are rather wide and of fairly thick glass, they should be rotated in the flame to prevent local overheating and cracking. Gently pull off the tops.

Heating methods of checking the seals are not permissible. Use the vacuum-dye technique.

Gross errors in distribution can be detected easily by inverting the ampoules and shaking the powder into the relatively narrow necks. This must not be done until the necks are cold. Slight variations may be ignored, often they are due to differences in the internal diameters of the necks or the shapes of the seals.

### 5 Sterility Test

#### NEUTRALISATION

The drug is a barbiturate. A large volume of broth is required, 50 ml is suggested.

#### SPECIAL FEATURES

Precipitation may obscure growth and necessitate subcultures.

(continued)

**Exercise 26 2 continued****SAMPLE SIZE**

The weight in the container is 100 mg. Therefore, at least 50 mg must be used for each test. It is convenient to use half the ampoule contents.

**MEDIA AND CONTROLS**

The points discussed in the previous exercise apply. The powder for the controls can be obtained from a second ampoule (as will be assumed in this case) or from the remainder in the jar.

**TABLE OF RESULTS**

(See Table 26 2)

**TECHNIQUE**

Loosen the caps of the media bottles.

Aseptically open two ampoules (one for the control) and cover them with sterile tubes or metal caps.

Pick up one and add to it about 2 ml of broth from the aerobic bottle. Dissolve or suspend the powder by taking the liquid into the pipette several times.

Transfer half back to the aerobic bottle. Shake to mix.

Transfer the other half to the bottom of the anaerobic bottle without introducing air. Rotate gently to mix.

Inoculate the controls in the same way from the other ampoule. Allow time for diffusion and then add appropriate organisms.

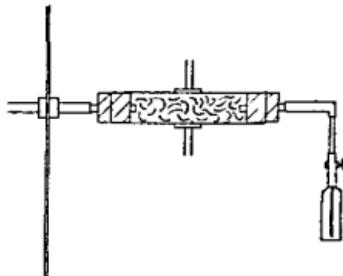
For further details see the testing of soluble powders in small ampoules in chapter 25.

When the tests have been passed, pack the ampoules and supply with them a box of six 2-ml ampoules of Water for Injection free from Carbon Dioxide that have been sterilised by autoclaving.

**Nitrogen Filling of Powder Ampoules**

This is occasionally necessary, e.g. for Neoarsphenamine (Sterile) used for making Neoarsphenamine Injection B.P.C.

The nitrogen must be sterile, and this is conveniently ensured by passing the gas through a tube about 8 in. long and 1 in. diameter carefully packed with non absorbent cotton wool and sterilised in a dressings steriliser. For use, the tube is attached at one end to the nitrogen supply via a connexion in the side of the tall sealing screen, and at the other to a dust hood and filling needle (Fig. 26 19). The unit is used in the same way as for air displacement from liquid ampoules but the flow rate must be much less because a powder is easier than a liquid to blow out of an ampoule. Seal each ampoule immediately after displacement.



*Fig. 26 19 NITROGEN FILLING UNDER ASEPTIC CONDITIONS*

Table 26.2  
Sterility Test on 200 mg ampoule of Phenobarbitone Sodium

	Label	Medium	Type	Neutralization by	Organisms— 1 ml of a 1 in $1 \times 10^8$ dilution of an 18–24 hr culture of	Incubation	Result	
							Expected	Observed
							Test	Subculture
504 Test	Aerobic TA	100 mg	50	Nutrient broth	Dilution	5 days at 37°C (Subculture if necessary)	—	—
	Anaerobic TN	do	do	Cooked meat	do	do	—	—
Main Control	Aerobic CA	do	do	Nutrient broth	do	<i>Staphylococcus</i> <i>aureus</i>	do	+
	Anaerobic CN	do	do	Cooked meat	do	<i>Clostridium</i> <i>histolyticum</i>	do	+

The above assumes the use of separate aerobic and anaerobic media.

### III. PREPARATION OF INJECTIONS FROM STERILE THERMOLABILE SOLIDS

#### 1 FROM SOLIDS IN AMPOULES USING AN AMPHOULE OF SOLVENT

The nature and dose of the medicament and the nature and volume of the solvent are carefully checked

If the ampoules require filing this is done at the base of the neck. Their surfaces are swabbed with 75 per cent ethyl or isopropyl alcohol and they are left to stand while the syringe is prepared. The swabbing is primarily to remove dust but a limited lethal effect on vegetative organisms can be expected

The syringe is assembled (see chapter 24) and left on its side with the nozzle end within the tube used for its sterilisation

Any solvent or powder in the necks of the ampoules is sharply shaken into the body

The powder ampoule is picked up, held almost horizontally and its neck is removed, keeping the fingers well away from the point of fracture. Then the ampoule is placed under an inverted sterile beaker or gallipot

The liquid ampoule is opened in the same way, the syringe is picked up and the required volume withdrawn. The empty ampoule is put down and the powder ampoule picked up and held almost horizontal while the solvent is added

Unless the medicament is very sensitive to oxidation (e.g. the arsphenamines) or carbon dioxide (the barbiturates) the ampoule is gently agitated to hasten solution

#### 2 FROM SOLIDS IN MULTI-DOSE CONTAINERS

The essential differences are—

The top of the bottle is swabbed and allowed to dry

A sterile plugged needle is passed through the cap to allow escape of air and facilitate introduction of the solvent.

The solvent can be obtained from an ampoule (preferably), a multi-dose container (in which case another plugged needle is required for this—to admit air during solvent withdrawal) or (in a pharmaceutical department) a McCartney bottle

If necessary, the containers of solutions prepared in this way are relabelled to indicate the storage requirements and life of the solution

Injections of antibiotics, e.g. benzylpenicillin, may be made in this way

The preparation of a large number of such injections is facilitated by using a distribution unit for

the solvent and replacing the air-escape needles by a device for vacuum removal of some of the air in the vials before filling. A branch containing an air filter is incorporated in the screen vacuum line and connected to its own needle or, via a two-way tap, to the tubing of the needle of the unit. In both cases the cannula of the unit must be replaced by a sharp needle

The unit is aseptically filled with solvent (see chapter 24). Then,

(a) If the branch has its own needle, this is passed through the rubber plug of the vial and, after gentle evacuation, the vial is removed and pushed on to the needle of the unit from which the required volume

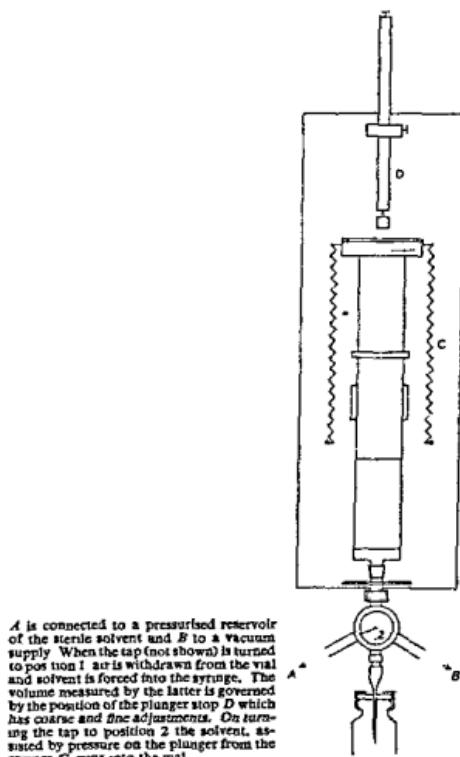


Fig. 26.20 MATBURN F 5 FILLING UNIT (DIAGRAMMATIC)

of solvent is then carefully added. Gentle evacuation avoids loss of powder by entrainment.

(b) If the needle of the unit is used, the vial is pushed on to it and the tap is turned first to the

evacuation and then to the filling position. The Matburn F.5 unit (Fig. 26.20) is of this type, it is also suitable for rapidly filling sterile solutions into empty sealed vials.

#### IV. PREPARATION OF STERILE DUSTING POWDERS CONTAINING THERMOLABILE MEDICAMENTS

##### EXERCISE 26.3

*Prepare four 5 G quantities of a surgical dusting powder containing 5,000 units per gramme of benzylpenicillin in sulphathiazole*

###### 1 Formula

	Amounts prescribed	Amounts used
Benzylpenicillin	5,000 units	100,000 units
Sulphathiazole	to 1 G	to 20 G

Microcrystalline sulphathiazole gives a more free flowing powder than powdered sulphathiazole

###### 2 Calculation

Vials containing 100,000 units of benzylpenicillin are available and it is convenient to use the complete contents of one of these for this exercise.

However, if a smaller number of units had been required the appropriate weight would have been calculated from the strength given on the vial.

E.g. If 20,000 units are needed and the strength on the vial is 1,670 units/mg,

$$\text{the number of mg required} = \frac{20,000}{1,670} = 12 \text{ mg}$$

This is unweighable

Therefore, weigh 50 mg and mix it with 450 mg of sulphathiazole. 10 mg of this mixture is equivalent to 1 mg of penicillin.

Therefore, 120 mg of the mixture is required.

###### 3 Label

Assume supply other than on a prescription

5 G
<u>PENICILLIN</u>
<u>AND SULPHATHIAZOLE</u>
<u>DUSTING POWDER</u>
5 000 units of benzylpenicillin per gramme of sulphathiazole
POISON
Store in a cool place
To ensure sterility use on one occasion only
Aseptically dispensed 19.8.63 (1)
Name _____

The word 'Poison' would be in red because sulphathiazole is a Schedule 1 poison

Dusting powders must always be kept dry. In this case it is particularly important because penicillin rapidly loses activity if it becomes damp.

'Use on one occasion only' is recommended because accidental contamination may occur during opening and closing of the container.

#### 4 Preparation

Strict aseptic technique is necessary

##### STERILE APPARATUS AND MATERIALS REQUIRED

Manufacturer's pack of benzylpenicillin containing 100,000 units

Exactly 20 G of microcrystalline sulphathiazole, dried and then sterilised in a hot air oven (see chapter 22). A double paper envelope is a convenient pack.

Glazed porcelain mortar and pestle. Since the mouth of a mortar is large and the mixing of the two powders takes some time it is useful to provide some form of cover. A piece of aluminium foil pulled well down over the sides, before the mortar is wrapped in paper, is one possibility but allowance must be made for the delay in heating-up caused by the reflection of heat from this.

Powder dredgers (see chapter 22)—sterilised by dry heat, the vial separately from the metal caps.

##### METHOD

Separate the aluminium sealing ring from the penicillin vial with a pair of sterile, but old, pointed scissors.

Undo the string round the paper cap on the dredger and loosen the screw cap on the jar containing its closures.

Unwrap the mortar, leaving the foil cover in position.

Unwrap the pestle and slip it under the foil into the mortar.

Hold the vial nearly horizontal, remove the rubber plug with flamed forceps and tip the contents into the mortar, holding the foil above the mouth meanwhile.

Remove the sulphathiazole from the outer envelope. Cut off a corner from the inner one with sterile scissors. Pour into the mortar an amount approximately equal to the weight of penicillin and mix well using the foil to give as much protection as possible. Continue additions of amounts of sulphathiazole approximately equivalent to the amounts of mixture in the mortar until all has been incorporated. Between additions keep the envelope on a sterile tile.

Weigh 5 G of the mixture into a paper envelope, lift the paper cap of the first dredger and transfer the powder to it through a wide-stemmed funnel (a good alternative is an inverted glass dust hood, as used in a distribution apparatus, because this has a wide stem through which the powder passes quickly). Replace the paper cap. Tough paper envelopes of the types used for the sterilisation of dressings and surgical equipment are useful containers for the aseptic weighing of relatively large amounts of powder, they give good protection during weighing and are easy to handle afterwards. Repeat with the other dredgers.

Using flamed forceps tightly screw the sister and protective caps on each vial, removing the paper caps at the last possible moment.

(continued overleaf)

*Exercise 26.3 continued*

Seal with an aluminium foil cap. Plastic rings or caps are inadvisable because they have to be applied wet, and penicillin must be protected from moisture. It is for this reason that the powder is packed in a dredger and not in the more moisture permeable paper envelopes often preferred for surgical powders.

**NOTES**

It will be noticed that the powder was not made up to 20 G. Instead, 20 G of sulphathiazole was added to the penicillin. This is permissible because the error is so small (100,000 units is approximately equal to 60 mg) and dosage with a dusting powder is not precise. It also avoids the aseptic weighing of a large amount of powder. Where a trituration is necessary to obtain the correct weight of penicillin the sulphonamide is not weighed before sterilisation because a separate amount is necessary for the trituration. In the example given under 'Calculation' above, the 450 mg could be weighed in a boat and the 19.88 G (20 G - 120 mg) in a bag. The triturate would be mixed with the rest of the sulphathiazole in a second mortar.

**5 Sterility Test**

Assume that one of the dredgers can be used.

**NEUTRALISATION**

Sulphathiazole—by using media containing para aminobenzoic acid  
Penicillin—by the addition of penicillinase

**SPECIAL FEATURES**

Sulphathiazole is insoluble. Therefore shaking is, and subculturing may be, necessary.

**SAMPLE SIZE**

At least 50 mg will be used. This will not be exceeded because of the turbidity produced by the sulphathiazole.

**MEDIA**

Nutrient broth and cooked meat medium containing 10 mg/100 ml of PABA are commonly used. Bottles are desirable because the aerobic containers must be shaken.

It is easier to carry out the neutralisation of the penicillin in a water insoluble powder after the samples have been added to the culture media. Pre-neutralisation presents the problem of accurately sampling the suspension of sulphathiazole in penicillinase.

The volume of penicillinase for each tube is calculated as follows—  
Assume that 1 ml. will neutralise 500 mg, i.e. at least 800,000 units of penicillin. (The B.P. states that 150 mg of benzylpenicillin is approximately equivalent to 250,000 units.)

The sample size is 50 mg, but calculate for twice this amount to allow for inaccuracy in estimating the weight, because this is normally done by eye.

$$100 \text{ mg contains } \frac{5,000}{10} = 500 \text{ units of penicillin}$$

This will be neutralised by  $\frac{500}{800,000}$  = approximately 0.0007 ml of penicillinase

0.1 ml is the smallest convenient volume to measure and it makes lavish allowance for the extra amount of enzyme needed for small unitages of penicillin.

The volumes of media are influenced by the volume of penicillinase if this contains a phenolic bactericide, in this case 10 ml gives more than adequate dilution.

#### TABLE OF RESULTS

This would be based on the example given on pp 460 and 461

#### METHOD

The penicillinase is added from a graduated pipette to all the containers in which it is required.

The samples are transferred with a flamed spatula.

The aerobic bottles are well shaken and the anaerobic ones tapped gently to encourage the powder to fall to the bottom.

They are incubated for an hour at 30°C, to ensure neutralisation of the penicillin, before adding the organisms to the control tubes and subsequent incubation at 37°C.

The penicillinase should be tested for sterility and freedom from inhibitory effect.

## V. PREPARATION OF EYE OINTMENTS

The eye ointments of the British Pharmacopoeia (Atropine, Hyoscine and Sulphacetamide) are made by aseptically incorporating the medicament into a previously sterilised base.

The medicament is not required to be sterile but

none of the substances listed above is likely to support bacterial growth in the solid state and, therefore, significant contamination from this source is improbable.

### EXERCISE 264

#### Prepare 5 G of Atropine Eye Ointment B.P.

##### I Formula

###### (a) Base

	B.P. amounts	Amounts used
Liquid paraffin	10 G	1 G
Wool fat	10 G	1 G
Yellow soft paraffin	80 G	8 G

The large excess is to allow for manipulation losses. It would be proportionally smaller for a larger quantity.

###### (b) Ointment

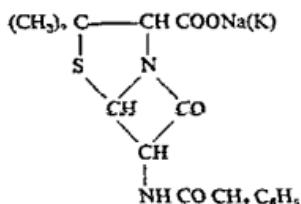
	B.P. amounts	Amounts used
Atropine sulphate	1 G	60 mg
Sterile base	to 100 G	to 6 G

(continued overleaf)

## VL PENICILLIN AND ITS PREPARATIONS

Although a detailed discussion of all the substances and preparations that require aseptic handling is impracticable, penicillin and its products merit special consideration because they have been a major stimulus to the development of aseptic technique.

Penicillin is a synonym for Benzylpenicillin B.P. This is the potassium or sodium salt of an antimicrobial acid (see Cooper and Gunn, 1957). It has the formula:



### STABILITY FACTORS

#### A Dry Salts

The dry salts are very stable. For example, not more than 10 per cent of the activity of Benzylpenicillin B.P. is lost on heating in an open container for 4 hr at 105°C.

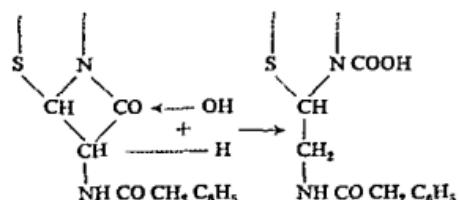
Provided they contain less than 1 per cent of moisture their potency is retained for many years at room temperature. Therefore, they must be packed in moisture-proof containers (see chapter 21).

Anhydrous preparations, such as ointments, tablets and lozenges, are stable for several months if stored in such containers in a cool dry place.

Both salts are hygroscopic, the potassium less than the sodium. This increases the need for good closures.

#### B Aqueous Solutions

Rapid hydrolysis occurs in aqueous solutions. This opens the  $\beta$ -lactam ring of the molecule to produce dibasic penicilloic acid.



Consequently—

- (a) Solutions have a very short life even when refrigerated. Unbuffered injections, kept at 2 to 10°C, must be used within 7 days.
- (b) Sterilisation by filtration is impracticable.

The stability of solutions is influenced by

#### 1 TEMPERATURE

The hydrolysis rate increases with rise of temperature. For example, Clapham (1950) found that solutions containing 100 000 units of benzylpenicillin per ml lost 22 to 50 per cent of their potency after 1 day at 24°C.

Therefore,

- (a) Heat sterilisation of solutions is impossible and, since filtration has also been excluded, aseptic technique must be used to ensure sterile preparations.
- (b) Low temperature storage is advisable. Unbuffered Benzylpenicillin injection must be used within 24 hr if kept at temperatures approaching 20°C.

#### 2 pH

Optimum stability is shown within the pH range 6 to 6.5. Because the hydrolysis products are more acid than benzylpenicillin the pH falls, with consequent increase in the rate of destruction and further drop in pH, this continues until all the penicillin is destroyed. Loss of activity can be significantly reduced by adding a buffer to maintain the pH between 6 and 7. Citrate is commonly used. Clapham (1950) obtained the results shown in Table 26.3, using solutions of benzylpenicillin (potassium salt) containing 100 000 units/ml, some of which were unbuffered while others contained 4 per cent w/w of anhydrous sodium citrate.

Notice that the citrate concentration is percentage weight in weight of the amount of penicillin used. An isotonic solution of benzylpenicillin contains

Table 26.3

Temperature (°C)	Time (days)	Storage conditions		Potency retained %
		Unbuffered	Buffered	
37	1	14	90	
24	3	11	90	
4	14	20	90	

rather less than 100 000 units/ml. The B P injection, when no dose is prescribed, contains 250,000 units/ml, i.e. is markedly hypertonic. The addition of 4.5 per cent weight in volume of sodium citrate would increase this and make the injection very painful. Buffered Benzylpenicillin Injection B P may be stored for 14 days at 2 to 10°C and 4 days at temperatures approaching 20°C.

### 3 CONCENTRATION

Concentrated solutions deteriorate most rapidly, presumably because the pH fall due to hydrolysis is greater than in weaker solutions. The Bacteriostatics subcommittee of the Conference on the Control of Antibiotics (1954) obtained data illustrating this, shown in Table 26.4

Table 26.4

Potency (units/ml)	Citrate (% w/v)	Potency loss after 4 days at 25°C %
100 000	4 to 5	0
500 000	5	25 approx

However, at 4°C the concentrated solutions showed no loss of potency in 8 days. Therefore, the B P storage directions give an adequate margin of safety for solutions of 250,000 units/ml—the strength recommended when no dose is given.

### 4 PENICILLINASE

Penicillinase destroys penicillin by hydrolysis. The optimum pHs for penicillin stability and penicillinase activity are approximately the same and, therefore, pH adjustment cannot be used to inhibit the enzyme. Its activity in penicillin preparations is prevented by—

- (a) Issuing a sterile product and/or
- (b) Including a bactericide
- (c) Refrigeration (injection) or storage in a cool place (other preparations)
- (d) Where possible, dispensing an anhydrous preparation (e.g. the eye ointment) in which penicillinase producing contaminants are unlikely to multiply.

### 5 TRACES OF HEAVY METALS

Traces of copper, lead, zinc, mercury and, possibly, other heavy metal ions catalyse the breakdown of the sulphur-containing ring in penicillin. Sequestering agents have been used to reduce instability from this cause (see chapter 20). Contact with heavy metals is minimised by using aluminium for the sealing rings

of vial closures and stainless steel or plastic cannulae for filling.

### 6 RUBBER ADDITIVES

Types and grades of rubber that have been tested for freedom from harmful additives (see chapter 21) are necessary for the closures of penicillin vials and the tubing used on filling units. Good quality autoclavable polyvinyl chloride is suitable for the latter.

### 7 OXIDISING AGENTS

These cause rapid destruction particularly if trace metals are present. Oils and fats used for ointments and oily injections must be free from rancidity because this is due to the oxidising activity of organic peroxides.

## PENICILLIN PREPARATIONS IN WHICH ASEPTIC TECHNIQUE IS USED

### 1. Injection

The dispensing of antibiotic injections is controlled by the Therapeutic Substances Regulations. Pharmacists, other than hospital pharmacists, who wish to do this work must obtain a licence from the Ministry of Health. However, this restriction does not apply to the supply of an antibiotic in its original unopened vial together with a separate container of solvent, because no manufacturer is involved. Applicants for a licence must satisfy the Ministry that they have adequate accommodation, equipment and staff. For full information see Antibiotics (1952).

In general the preparation of benzylpenicillin solutions follows the principles already outlined for the aseptic dispensing of injections from thermolabile solids but two special precautions apply:

- (a) If there is any risk of the solution touching the skin rubber gloves should be worn to prevent sensitisation (q.v.).
- (b) Metal contact should be minimised.

When the caps of sealed vials have to be penetrated the use of metal cannot be avoided altogether but brief contact with a stainless steel needle or cannula is not harmful. All glass syringes are advisable. When ampoules or open vials are filled from a unit, a glass or nylon cannula can be used.

Benzylpenicillin is supplied sterile in antibiotic vials containing amounts ranging from  $10^5$  to  $10^7$  units. Vials containing the antibiotic ready mixed with dry buffer are also obtainable.

Occasionally it may be necessary to supply a buffered multi-dose solution using buffer free penicillin, as in Exercise 26.5.

**EXERCISE 26.5**

*Prepare 5 ml of Benzylpenicillin Injection solution containing approximately 200,000 units/ml*

A mega (million) unit vial would be used.

1 mega unit weighs approx. 600 mg

Assuming the use of approximately 5 per cent w/w of sodium citrate as a buffer,  $\frac{600}{100} \times 5 = 30$  mg are required.

This must be in solution because it must be added without removing the vial closure

As this is a multi-dose injection a bactericide is necessary, and the most satisfactory substances appear to be phenylmercuric nitrate (0.001 per cent) and phenol (0.5 per cent). For buffered preparations the former has a slight advantage because if phenol preserved citrate-buffered injections are left at room temperature crystals of phenylphenaceturate deposit (Woodard, 1952).

It is convenient to have ready prepared an autoclaved solution of 0.001 per cent phenylmercuric nitrate containing 6 mg of sodium citrate/ml, of which 5 ml can be aseptically added to the vial, in the usual way, to give a buffered and preserved solution of the correct strength.

Preferably the bactericide-citrate solution should be packed in ampoules because of the absorption of phenylmercuric nitrate by rubber. Careful watch must be kept for spicule formation.

When sodium ethylenediamine tetra acetate (0.2 per cent) is used as a sequestering agent neither of the above bactericides is suitable. Swallow (1952) has suggested domphen bromide (0.02 per cent) instead.

For further information on the preparation of penicillin injections see Carr and Wing (1951), Coulthard, Fawcett, Lewis and Sykes (1951) Hadgraft, Hopper and Short (1951), and Hobbs, Livingstone, Reece and Woodard (1952).

The label must comply with the Therapeutic Substances Regulations

5 ml
<u>BENZYL PENICILLIN</u>
<u>INJECTION B.P.</u>
200 000 units per ml
Store at between 2 and 10°C and use within 14 days
<u>Buffer</u> 0.6% w/v Sodium citrate
<u>Bactericide</u> 0.001% w/v Phenylmercuric nitrate
<u>Expiry date</u> 15/11/64 <u>Licence</u> 124
<u>Aseptically prepared</u> 1/11/64 (4) <u>Name</u>

It has been assumed that cold storage is possible. This should always be confirmed and if a refrigerator is not available the storage directions must be amended to 'Store in a cool place and use within 4 days' and the expiry date must be modified accordingly.

The licence of the manufacturer of the antibiotic and his batch number are also required. It is less confusing if these are put on a separate label.

Occasionally, intrathecal injections of penicillin are used, 200,000 units in 5 ml being an average strength. A special intrathecal grade of penicillin, prepared with scrupulous care to remove all traces of foreign matter, is available. It is supplied in ampoules, and the injection is made immediately before use. No bactericide may be added.

## 2 Eye Drops

Usually penicillin eye drops are made to the following formula:

Benzylpenicillin	15,000 units
Sodium citrate	0.5 gr (0.5 per cent)
Solution for Eye Drops	to 110 ml

Aseptic technique and a sterile container are necessary and it is preferable, but not obligatory, to use sterile solvent and equipment.

Buffered Solution Tablets of Penicillin B.P.C., each containing 15,000 units of penicillin and 0.5 gr of sodium citrate, are a convenient source of the ingredients.

About 80 ml of solvent is aseptically poured into a covered two-drachm measure that is either sterile or well rinsed with solvent. A tablet is removed from the tube with flamed forceps and added to the solvent. The tube is immediately closed to protect the remaining tablets from moisture. The tablet is dissolved by stirring with a flamed but cool glass rod and the solution is passed by gravity through a small grade-3 sintered glass filter into the bottle and made up to volume through the filter. To facilitate the latter the container is graduated before sterilisation on a side that will be covered by the label eventually. The cap is put on aseptically and its junction with the bottle sealed with a plastic ring. The label must carry instructions for storage—Keep in a cool place and use within a week'.

The buffered tablets are not intended for injection. They must be kept cool and packed in a container that excludes moisture. Unused tablets must never be handled with the fingers.

(For further information on eye drops see chapter 13.)

## 3. Eye Ointment

The normal strength is 2,000 units/gramme of sterile Eye Ointment Base B.P. Strict aseptic technique is used. As the medicament is sterile, the product

should comply with sterility tests (Compare other eye ointments). Cool dry storage is necessary.

## 4 Dusting Powders

An example is discussed on p. 506.

## DEPOT INJECTIONS OF PENICILLIN

Most prolonged action injections of penicillin contain procaine penicillin, a relatively insoluble complex obtained from the interaction of benzylpenicillin and procaine hydrochloride. This is issued in four forms—

- 1 As the dry solid e.g. Procaine Penicillin Injection B.P.
- 2 As the dry solid mixed with dry benzylpenicillin, e.g. Fortified Procaine Penicillin Injection B.P.
- 3 As a ready-made aqueous suspension, e.g. Sterile Procaine Penicillin G Suspension U.S.P.
- 4 As ready-made oily suspension, e.g. Sterile Procaine Penicillin G with Aluminium Stearate Suspension U.S.P.

All these may contain adjuncts such as dispersing, suspending and buffering agents and bactericides. The problems connected with the formulation and preparation of injectable suspensions (see chapter 20) necessitate very careful control measures, and therefore manufacture is largely confined to pharmaceutical houses. Normally, the addition of sterile water to forms 1 and 2 is the only dispensing involved, allowance must be made for the comparatively large volume of insoluble powder but the manufacturer's literature gives guidance on this. The vial is immediately shaken afterwards and labelled with a clear instruction to shake well before use. Cetrimide 0.025 per cent is often used as a bactericide (see Sargent, 1958).

(Further information on the formulation of depot penicillin preparations will be found in the references in the suspension section of chapter 20 and in Hastings (1956) and Levin (1953))

## REFERENCES

- ANTIBIOTICS (1952) *Antibiotics Properties and uses* 2nd Ed. The Pharmaceutical Press, London.
- AVIS, R. E. and GERSHENFELD, L. (1955) Bacteria excluding filters for oils *J Amer pharm Ass, Sci Ed* 44, 682-687
- BACTERIOSTATIC SUBCOMMITTEE OF THE CONFERENCE ON THE CONTROL OF ANTIBIOTICS (1954) Stability of penicillin solutions *Pharm J* 172, 229-231
- BARFIELD, J C (1955) Sterilisation by filtration. *Pharm J* 174, 9
- BRITISH STANDARD 1752 1963 *Laboratory sintered or fritted filters* British Standards Institution, London.
- BROWNE, H H (1942) Changes in reaction caused by filtration through Seitz filters *J Bact* 42, 315-316
- CARR, T and WING, W T (1951) The stabilisation of solutions of penicillin *Pharm J* 167, 63-65
- CLAPHAM, P C H V (1950) The stability of penicillin. *Pharm J* 165, 126-129
- COOPER, J W and GUNN, C. (1957) *Tutorial Pharmacy* 5th Ed Pitman, London. 482-491
- COULTHARD, C E, FAWCETT, R, LEWIS, D G and SYKES, G (1951) The stability of penicillin solutions at normal and higher temperatures *J Pharm Pharmacol* 3, 748-754
- DUBOS, R J (1949) *The Bacterial Cell* Harvard University Press, Cambridge, Mass., U.S.A
- ELLIOTT, J R and RUTTER, J S (1952) Small scale sterilising by filtration. *Alchemist, Leeds* 16, 10-16
- HADGRAFT, J W, HOPPER, C G and SHORT, P (1951) Stability of aqueous solutions of crystalline penicillin sodium. *Pharm J* 167, 13-15
- HASTINGS, J H. (1956) Aqueous suspensions of procaine penicillin. *Pharm J* 177, 141-142
- HOBBS, R J, LIVINGSTONE, J I., REECE, J and WOODARD, W A. (1952) Preliminary observations on the stabilisation of penicillin solutions with hexamine. *J Pharm Pharmacol* 4, 911 916
- LAX, E J (1938) A bacteria proof filter unit. *Pharm J* 141, 4
- LEVIN, R. (1953) Studies in the deterioration of aqueous solutions and dispersions of procaine benzylpenicillin *J Pharm Pharmacol* 5, 917-926
- LIGHTBOWN, J W (1962) The sterility testing of antibiotics Proceedings of the 7th International Congress for Microbiological Standardisation. Livingstone, Edinburgh 220-225
- MARTIN, E W and COOK, E F (1961) *Remington's Practice of Pharmacy* Mack Publishing Co, Easton, Penn., U.S.A
- MILLIPORE CORPORATION (1961) *Millipore Technical brochure* of the Millipore Filter Corporation, Bedford, Mass., U.S.A
- MILLIPORE CORPORATION (1963) *Sterilizing filtration and sterility testing* Millipore Filter Corporation, Bedford, Mass., U.S.A
- OXOID MANUAL. (1961) *The Oxoid Manual of Culture Media* The Oxoid Division of Oxo Ltd., Thames House, E.C.4
- ROYCE, A and SYKES, G (1950) Aspects of sterilisation by candle filtration *Proc Soc appl Bact* 13, 146-151
- SARGENT, C. L (1958) Bacteriostats for parenteral injections of procaine penicillin *Pharm Weekbl* 93, 81 (Abstracted in *J Pharm. Pharmacol* (1958) 10, 396)
- SMITH, G and MITCHELL, J I (1962) A filter syringe for small scale sterilisation. *Pharm J* 188, 337-338
- SMITH, L C. P (1944) Sintered glassware, its manufacture and use *Pharm J* 152, 110-111
- SUTARIA, R. H. and WILLIAMS, F N. (1962) A new approach to the preparation of ophthalmic solutions *Pharm J* 188, 91-92
- SWALLOW, W (1952) Solutions of crystalline penicillin *Pharm J* 168, 467-468
- SYKES, G and ROYCE, A. (1950) The removal of bacteria from oils by filtration. *J Pharm Pharmacol* 2, 639-647
- SYKES, C H. (1958a) Sterile dispensing and the hospital pharmacist Part 2 Filtration sterilisation *M and B Pharmaceutical Bulletin* 7, 50-52
- SYKES, C H. (1958b) The testing of 5 on 3 sintered glass filters *M and B Pharmaceutical Bulletin* 7, 62-64
- WINDLE TAYLOR, E., BURMAN, N P and OLIVER, C W (1953) Use of the membrane filter in the bacteriological examination of water *J appl Chem* 3, 233 240
- WOKES, F (1936) An improved bacterial filter *Pharm J* 136, 313
- WOODARD, W (1952) Recent developments in the pharmacy of the antibiotics *J Pharm Pharmacol* 4, 1009-1046
- WYLLIE, D M (1955) Filtration by centrifugal force *Pharm J* 175, 492

## Gaseous Sterilisation

THE sterilisation processes described so far are either unsuitable or not ideal for—

- Thermolabile solid medicaments* Aseptic precipitation or crystallisation from a sterile solution is not a method that a hospital pharmacist for example, could conveniently use
- Thermolabile equipment*, e.g. articles of plastic, electrical diagnostic equipment, delicate rubber items and blankets

For equipment, sterilisation with a liquid disinfectant is possible if the item is not attacked by the

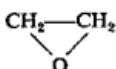
bactericide or its solvent, but removal of all traces of residual chemical is difficult and may necessitate aseptic washing, followed by aseptic drying or use of the article while still wet

A method of greater application is sterilisation with a chemical in the gaseous state Formaldehyde was once widely used, and new materials such as beta propiolactone are under constant investigation but, at present, ethylene oxide is the only compound of outstanding importance in the pharmaceutical and medical fields

### ETHYLENE OXIDE

#### General Properties

Ethylene oxide is the simplest cyclic ether and has the structure



At room temperature it is a colourless gas with a characteristic ethereal odour. It can be liquified easily and the liquid boils at approximately 10.8°C. Water and organic solvents dissolve it readily.

Concentrations greater than 3 per cent in air are highly inflammable—with explosive violence in an enclosed space. This can be overcome by—

1 *Admixture with an Inert Gas* Mixtures containing 1 part of ethylene oxide with 9 parts of carbon dioxide or certain halogenated hydrocarbons are entirely safe with any proportion of air.

A mixture known as Cryoxide is used extensively in America. This contains 11 per cent w/w of ethylene oxide, 79 per cent of trichlorofluoromethane (known in this country as Arcton 11) and 10 per cent of dichlorodifluoromethane (Arcton 12). In Eng-

land the mixture commercially available contains 10 per cent of ethylene oxide and 90 per cent of Arcton 12.

Carbon dioxide is less popular because it has a very high vapour pressure—about 54 000 mm Hg at 30°C. The values for Arctons 11 and 12 are approximately  $\frac{1}{3}$  and  $\frac{1}{2}$  of this respectively. Consequently, at any particular working pressure, there is less ethylene oxide in the vapour when carbon dioxide is used. In addition, stouter containers (cylinders) are needed. Cryoxide, which has a high percentage of Arcton 11 can be packed in light (aerosol type) cans.

2 *Using the Gas in the Absence of Oxygen* Exposure is carried out at subatmospheric pressure in a previously evacuated steriliser. This method is preferred in Germany (Mayr, 1961), a mixture containing 90 per cent ethylene oxide and 10 per cent carbon dioxide is used in a chamber from which at least 95 per cent of the air has been removed. The carbon dioxide reduces but does not eliminate the inflammability of the gas which, therefore, should be used only in equipment specially designed for it.

### Antimicrobial Activity

Ethylene oxide is a powerful alkylating agent and its antimicrobial activity is probably due to alkylation of the sulphhydryl, imino, carboxyl and hydroxyl groups of proteins and other important cell constituents (see Bruch, 1961). For example—



The alkyl groups are very firmly bound, which may explain the bactericidal effect of ethylene oxide.

It is active against all micro-organisms and there is only a small difference between the concentrations necessary to kill vegetative bacteria and spores in the same time. The ratio is 1.5 much less than the  $10^3$  to  $10^4$  ratio for liquid disinfectants. One of the most resistant organisms is *Bacillus subtilis var globigii* (also known as *Bacillus globigii* and *Bacillus subtilis var niger*) the spores of which are often used for testing ethylene oxide sterilisers.

### Factors Influencing Efficiency

#### 1 CONCENTRATION

Usually, concentrations are expressed in mg/litre because the sterilisation rate depends on the partial pressure of ethylene oxide, which is determined by the amount in a specified volume of the chamber atmosphere. Concentrations used for sterilisation range from 200 to 1,000 mg/litre and, since the dilution coefficient (see 'Sterility Testing') is approximately 1, if the concentration is doubled the exposure time is approximately halved. One pharmaceutical house in this country regularly and successfully uses 200 mg/litre (10 per cent v/v) for 16 to 18 hr overnight at room temperature (20°C approx.) (Royce, 1959). An American firm that manufactures ethylene oxide sterilisers recommends, for most purposes, exposure to 850 to 900 mg/litre for 3 hr, or 450 mg/litre for 5 hr, at 45°C (Perkins and Lloyd, 1961). For the sterilisation of hospital equipment Freeman and Barwell (1960) used 555 mg/litre for 14 hr at 30° to 40°C, this included a very large safety margin in the time factor (see later).

The adequacy of these treatments is confirmed by figures (Table 271) from Phillips (1961) for the

Table 271

Ethylene Oxide concentration (mg/litre)	Exposure time (hr)
88	10
442	4
884	2

exposures required to kill  $5 \times 10^6$  spores of *Bacillus subtilis var globigii* (dried on cloth) at 25°C.

Nevertheless, this data indicates a serious disadvantage of ethylene oxide sterilisation—its slowness.

#### 2 TEMPERATURE

Sterilisation can be achieved at room temperature, but a long time is necessary and, often, advantage is taken of the decrease in time with rise in temperature. Roughly, the exposure can be halved for each increase of 20°C. However, since gas sterilisation is used for thermolabile materials, very high temperatures are impractical and 60°C can be regarded as the upper limit.

#### 3 RELATIVE HUMIDITY

'Some moisture is necessary but a little is better than a lot.' This statement by Phillips (1961), the leading authority on ethylene oxide sterilisation, indicates the complicated effect of this factor.

The moisture content of the cells and the relative humidity of their immediate environment appears more important than the condition of the chamber atmosphere. Phillips has suggested that deficiency of water in the cells prevents ionisation of groups susceptible to alkylation which, therefore, cannot take place.

It is difficult to destroy organisms that have been excessively dehydrated, e.g. by exposure to extremely low humidities or to high vacua. Phillips has also suggested an explanation for this. Normally, proteins contain linkages involving water molecules; when dehydration takes place these are replaced by much stronger links that utilise potential alkylation points, which are then no longer available for combination with ethylene oxide. Extensive blockage of this kind makes organisms almost completely resistant.

Organisms dried on hard impermeable surfaces such as glass, plastics and metal are less easy to kill than organisms dried on materials that can absorb moisture, e.g. paper or cloth (Opsell, Hohmann and Latham, 1959, Royce and Bowler, 1961).

Phillips (1961) has shown that the optimum relative humidity for unprotected spores exposed on cotton patches is approximately 30 per cent. Sterilisation occurs 4 times faster than at near-saturation humidity. One suggested explanation is that in high humidities the ethylene oxide in the cells is diluted to below the most effective concentration.

Sufficient moisture must be present—

(a) During exposure, to produce optimum or near optimum humidity. Usually 50 per cent is

preferred because if 30 per cent is used and a fall occurs during exposure the efficiency of the process may be impaired, 50 per cent provides a safety margin and does not greatly increase the sterilisation time.

(b) *During the pre-vacuum period* Moisture is important at this stage because resistant organisms produced by vacuum dehydration can be restored to normal sensitivity only by complete wetting, or exposure to near-saturation humidities for several days (Phillips, 1961), neither is practicable in the middle of a sterilisation cycle.

#### 4 PHYSICAL PROTECTION OF ORGANISMS

Many workers, e.g. Royce and Bowler (1961), have reported occasional sterilisation failures and in some cases these have been due to enclosure of the organisms in gas impermeable deposits. Therefore, articles for gaseous sterilisation must be scrupulously clean.

Films of organic matter, such as blood or serum, give less protection than films containing a significant number of crystals, e.g. dried nutrient broth. The gas can penetrate the former if they are not very thick or hard but it is unable to reach organisms trapped within the individual crystals of the latter.

Abbott, Cockton and Jones (1956) found that certain bacterial spores remained viable inside crystals for long periods and could not be destroyed by greatly extended treatment with bactericidal gases although normal exposures would produce sterility when only the surfaces of the crystals were contaminated. Consequently, it is inadvisable to rely on ethylene oxide alone for the sterilisation of thermolabile powders and crystals. Aseptic precipitation or crystallisation from a sterile solvent is necessary to exclude viable organisms from the interiors of particles. Then, if desired, asepsis can be relaxed and any contaminants collected on the surfaces during the subsequent handling destroyed by terminal gas sterilisation.

Talc is sometimes sterilised by ethylene oxide. In this case surface sterilisation is adequate because this powder is insoluble in the body and, in any case, internal contamination of the particles of a finely insoluble substance is highly improbable.

#### 5 POWER OF PENETRATION

An outstanding advantage of ethylene oxide over other agents used for gaseous sterilisation is its powerful penetrating ability. Paper, fabrics and a number of plastics and rubbers are freely permeable. Therefore,

(a) *Post-sterilisation aseptic handling is eliminated because articles can be wrapped previously*

(b) *The method is suitable for—*

- (i) Powders in rubber-capped vials (Royce, 1959), provided sufficient time is allowed for entry and post sterilisation escape of the gas through the rubber.
- (ii) Talc in bulk, e.g. in paper sacks.
- (iii) Electrical diagnostic equipment. The gas readily penetrates the many crevices of this complicated apparatus.
- (iv) Eye-drop bottles (Summers, 1962).
- (v) Plastic or rubber tubing and gloves—part of the gas reaches the inner surfaces by passing right through the article (cf. *Steam Sterilisation of Rubber Articles*).
- (vi) Blankets.
- (c) *Residual gas escapes readily from inside wrappers after sterilisation.* Some plastic wrapping films are not entirely satisfactory. Ideally they should be permeable to—
- (i) *Ethylene oxide.* Polythene is excellent but certain polyesters are relatively impermeable (Bruch, 1961).
- (ii) *Air* (but not micro-organisms). To reduce the risk of bursting during the pre-vacuum (see use of nylon film in steam sterilisation).
- (iii) *Water vapour.* To ensure satisfactory humidity conditions inside the wrapper. Often this factor will not be very important because the low moisture requirement will be provided by the contents of the pack.

The superiority of cloths and many papers in these three respects should not be overlooked.

Penetrating power has its disadvantages. The gas is difficult to enclose and, because of its inflammability and toxicity, potential leakage points in the sterilisation chamber and ancillary apparatus must be very efficiently sealed.

#### 6 ABSORPTION

Many materials absorb ethylene oxide strongly. This has several effects, e.g.

(a) *Reduction of the gas concentration in the chamber atmosphere.* The amount and rate of absorption depends on the size of the load and the nature, thickness and surface area of the articles and their wrappings. Some examples of ethylene oxide absorption are given in the Table 27.2, they were determined by Royce (1959) after the materials had been in contact with a gas concentration of 200 mg/litre for 24 hr at 25°C.

Either the gas concentration or the exposure time can be increased to compensate for the loss. The

Table 27.2

Material	Amount absorbed (mg/G)
Polythene	2
PVC	19.2
Bakelite	0
Brown paper	6.1
Cardboard	10.4
Non-absorbent cotton wool	4.1
Red rubber closures	5.5
Neoprene rubber closures	15.2
Starch glove powder	10.5
Talc	0.5
Procaine penicillin	0.2
Water	25 to 30

former is more wasteful, particularly as absorption increases with gas concentration. With experience, appropriate additions to the time can be calculated from the size and nature of the load.

(b) *Because of the toxic effects of ethylene oxide the sterilised articles cannot be used until absorbed gas has escaped* Freeman and Barwell (1960) reported the liberation of bubbles of incompletely desorbed gas from the pvc tubing of a blood oxygenator.<sup>1</sup>

One method of desorption is 'airing', i.e. storage at ordinary temperature in a well ventilated room. A long time may be necessary. For example, Perkins and Lloyd (1961) found that samples of plastic and rubber tubing required 19 and 5 hours respectively while Royce (1959) allowed 7 to 10 days for complete clearance from powders in rubber capped vials.

Alternatively, dissipation can be hastened by applying a powerful vacuum immediately after sterilisation. Freeman and Barwell recommended a vacuum of 30 in. Hg for 2 hr, a time that includes a safety factor of 100 per cent.

(c) *The article receives an additional exposure during desorption* This provides a useful safety margin, particularly when airing is used.

#### Toxicity

The inhalation toxicity of ethylene oxide approximates to that of ammonia. High concentrations irritate the nose and, particularly, the eyes and may cause nausea and dizziness. With a well-designed leak proof steriliser that discharges the gas to the outside after sterilisation and is housed in a well-ventilated room the risk of reaching such levels is low. In unusually hazardous circumstances, e.g. in experimental work with prototype equipment, goggles and even a respirator should be worn.

A further source of danger is the vesicant action of the moist compound. Blisters have been caused by the vapour liberated during the wearing of inadequately aired rubber gloves and similar damage could follow the use of incompletely desorbed plastic and rubber articles in surgery.

#### Ethylene-oxide Sterilisers

At the time of writing, no steriliser is manufactured in this country but this will be remedied soon. Several types are marketed in Germany (Mayr, 1961) and in the USA (Perkins and Lloyd, 1961). The following is a general outline of the more important features of steriliser design and use.

#### 1 DESIGN

The features of suitable equipment include—

- An exposure chamber that is gastight and able to withstand high pressure and vacuum.
- A means of heating the chamber, e.g. a steam or hot water jacket or heating elements clamped to the outside.
- A baffled inlet for the gas mixture, usually at the bottom of the chamber. The baffle protects the contents from liquid ethylene oxide accidentally introduced. The liquid can badly damage certain plastics.
- A method for completely vapourising the gas mixture and warming it to the sterilisation temperature. In one arrangement (Wilkinson, 1960) the gas cylinder is connected to an expansion chamber which is followed by a heat exchanger consisting of a coiled tube surrounded by steam.
- A means of extracting air before, and the gas mixture after, sterilisation. A high efficiency pump (see 'High vacuum Steam Sterilisers') is desirable. It should discharge to the open air.
- A system for adding water to provide the right humidity.
- Provision for the admission of sterile air at the end of the process (see Steam Sterilisers).
- A safety valve and suitable indicators and recorders of pressure and temperature. Automatic control is advisable because of the significant effects of alterations in temperature and humidity.

As with steam sterilisation, the problem of accurate and sensitive measurement of humidity has not been satisfactorily solved.

#### 2 METHOD OF USE

The chamber is loaded.

Sufficient water is introduced to prevent vacuum dehydration of micro-organisms.

The door is closed and the temperature raised to sterilisation level unless exposure is to be carried out at room temperature.

The heat-exchanger is raised to a high temperature (100°C *ca*) because the gas mixture fails to well below room temperature as it leaves the cylinder.

The chamber air is removed by a pre vacuum of more than 28 in Hg (the B.P. recommends evacuation to about 10 mm Hg). On a small scale the gas may be used to displace the air (*cf.* Steam Sterilisation) but this method is wasteful and insufficiently reliable for large loads.

If necessary, more water is added to produce a satisfactory exposure humidity.

The warmed gas mixture is admitted until the correct pressure is reached.

#### *The exposure time is allowed*

The high vacuum is drawn again and held for a sufficient time to desorb all the gas from the contents. Alternatively, the vacuum may be broken as soon as 28 in Hg has been reached, and the articles desorbed at room temperature.

Sterile air is admitted and the load is removed.

Freeman and Barwell (1960) used a method similar to the above for plastic hospital equipment. They heated the chamber to 30° to 40°C, drew a pre-vacuum of approximately 30 in Hg, produced a relative humidity of 30 to 50 per cent, introduced sufficient gas mixture (10 per cent ethylene oxide and 90 per cent Arcon 12) to give a pressure of 5 lb/in<sup>2</sup> g (equivalent to 555 mg/litre of ethylene oxide), exposed for 14 hr (this allowed a large safety margin since sterility was produced in 2 to 4 hr) and desorbed for 2 hr.

In Germany, where the more dangerous 90 per cent ethylene oxide mixture is often used, sterilisation is carried out below atmospheric pressure to prevent escape of the gas. The low pressure is practicable because of the high ethylene oxide content of the mixture (see Mayr, 1961). With the low content mixtures used here and in America pressures in excess of atmospheric are essential if high ethylene oxide levels are required.

For details of (i) a bench autoclave modified for ethylene oxide sterilisation in a hospital pharmaceutical department, see Sutaria and Williams (1961) and (ii) simple techniques using vacuum desiccators, see Kelsey (1961) and Summers (1962).

#### **Control of the Process**

Methods similar to those for steam sterilisation are used.

#### **I DIRECT—STERILITY TESTING**

Two special problems arise—

(a) Experience shows that the probability of all the batch being unsterile if the process has failed (see p. 469) is lower than with steam sterilisation. Therefore, unless large numbers of samples are tested faulty processes may not be detected. The problem is made more difficult because with equipment loads it is rarely possible to test many items as this seriously reduces the number available for use.

(b) The testing of hospital equipment involves difficult manipulations that increase the risk of accidental contamination. Consequently, control is largely based on indirect methods.

#### **2 INDIRECT**

##### *(a) Instrumental*

As with sterilisation by dry heat and steam this method is of first importance, and when more light has been thrown on the optimum conditions for ethylene oxide sterilisation and better equipment for humidity control is available, the use of well-designed, well maintained, automatically-controlled sterilisers, providing temperature, pressure and humidity records that are regularly inspected will limit other forms of control to the development of new processes and occasional checks.

##### *(b) Bacteriological*

At present, regular bacteriological testing of ethylene oxide sterilisation is necessary because of the occasional failures reported.

Usually, test pieces, i.e. small pieces of a suitable material contaminated with bacterial spores, are placed in the least gas-accessible parts of a number of articles and containers situated in different regions of the steriliser. After exposure they are tested for sterility. Important features of test pieces are—

(i) The organism must have high and standard resistance to ethylene oxide. Spores of *Bacillus subtilis var. globigii* are commonly used.

(ii) The number of organisms must present a reasonable challenge to the process. A contamination level roughly equivalent to that normally present on the article would allow no safety margin and, because of the very small number of organisms on some articles (e.g. certain newly-manufactured plastics), a large number of pieces would be required to obtain a reliable result. Very high contamination levels, make unrealistic demands on the process and increase the probability of physical protection of some of the

cells. Usually, each piece is inoculated with  $10^6$  spores, all of which must be killed.

(iii) The organisms must be presented on a similar material to that of the articles undergoing sterilisation. Humidity considerations dictate that if the surface of the article is hard, e.g. a plastic, the test piece must not be a moisture absorbent material such as cloth and filter paper (see earlier, and Tattersall, 1961).

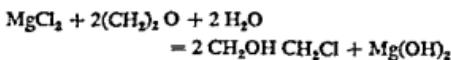
(iv) They should be of convenient size for sterility testing, i.e. easily handled with forceps and small enough to pass cleanly through the neck of a container of culture medium.

#### (c) Chemical

Royce and Bowler (1959) have invented a disposable physico-chemical indicator that they describe as an artificial macro micro-organism which changes colour when 'dead'. It is based on similarities between the destruction of organisms by ethylene oxide and the diffusion of a gas through a permeable membrane. If the relative humidity is satisfactory and the organisms are unprotected the death rate is proportional to the gas concentration. The diffusion rate of the gas through a defined area of a permeable membrane is also proportional to its concentration. Rise or fall of temperature respectively increases or decreases the rates of both death and diffusion.

The control is an ethylene oxide-permeable sachet containing an absorbent that changes colour as gas is absorbed. The size and thickness of the sachet (which is of polythene) and the quantity and strength of absorbent are arranged so that the specified colour is not produced until the sachet has been subjected to a lethal exposure (i.e. concentration and time product). The exposure on which Royce and Bowler based their device was the equivalent of 200 mg/litre for 16 to 18 hr at 20°C.

The absorbent is a saturated solution of magnesium chloride containing hydrochloric acid and bromophenol blue. Absorbed ethylene oxide is converted to ethylenchlorhydrin—



The magnesium hydroxide neutralises part of the hydrochloric acid and, therefore the pH gradually falls and the indicator changes from yellow to purple. Determinations of the times to kill bacterial spores (soil dust containing  $10^5$  spores/G) and cause the colour change showed good agreement. For example—

#### At constant temperature (20°C)

Ethylene oxide concentration	Time (hr) to kill the spores	change the sachet
1,000	4-7	4
500	7-8	8
200	16	19

#### At constant gas concentration (200 mg/litre)

Temperature (°C)	Time (hr) to kill the spores	change the sachet
20	16	19
40	5	6.5
50	4	4.5

Since changes in gas concentration and temperature have parallel effects on organism and sachet, no, or an incomplete, colour change in the latter indicates unsatisfactory sterilising conditions. The sachets cannot entirely replace bacteriological tests as indicators of satisfactory conditions because they do not allow for the humidity factor.

### Applications of Ethylene-oxide Sterilisation

#### 1 POWDERS

##### (a) Thermolabile

Because ethylene oxide cannot reach organisms inside solid particles it is advisable to restrict this method to the sterilisation of the surfaces of substances that have been aseptically precipitated or crystallised.

Ethylene oxide is highly reactive and when any new material is gas sterilised it must be examined for deterioration afterwards. Streptomycin, calcium chloride loses a large percentage of its potency but penicillin, tetracycline and erythromycin are unharmed.

The type of container is influenced by the length of storage before use and the sensitivity of the powder to moisture and air. Paper and plastic envelopes, rubber-capped glass bottles and polythene vials are possible choices.

##### (b) Thermostable

Bulk powders such as talc and Absorbable Dusting Powder B.P. present a considerable heat penetration problem in hot air sterilisation. Treatment with ethylene oxide is an effective alternative.

#### 2 EYE DROPS IN PLASTIC UNIT DOSE CONTAINERS

This application is described in the chapter on eye-drops.

### 3 EQUIPMENT

#### (a) Plastic

The steadily increasing use of plastics in hospitals was a major reason for interest in the medical applications of gaseous sterilisation. Commercial processes have been developed for catheters, syringes and needles. Other articles for which it is suitable include intravenous sets, prostheses, blood oxygenators, bottles and vials and polythene-covered stirrers for magnetic mixers.

Some plastics are damaged by ethylene oxide (Tessler, 1961). For example, the surface of polystyrene may become crazed. Contact with the liquid must always be avoided.

#### (b) Rubber

Fragile rubber articles survive more treatments by ethylene oxide than by steam. The method is used in many American hospitals for surgeon's gloves, Catheters, eye-drop bottles and stoppers for vials are more occasional applications.

#### (c) Electro-diagnostic

The low humidity and lack of damage to metals, rubbers and most plastics make ethylene oxide treatment suitable for cystoscopes, bronchoscopes, ophthalmoscopes, Geiger-Muller counters and similar delicate articles. The long time is a disadvantage because this type of equipment is too costly for large stocks to be held and, therefore, rapid turnover is desirable.

#### (d) Other Equipment

The method has been used for the sterilisation of mills before the grinding of sterile powders and is suitable for the occasional sterilisation of balances for aseptic technique.

### 4 BLANKETS

In recent years the disinfection of blankets has been the subject of many investigations (Extra Pharmacopoeia Supp., 1961a) because of the contribution made by blanket dust to the aenal flora of hospital wards and theatres. Ethylene oxide sterilisation is one method for which success has been claimed.

### 5 EXTERIOR OF AMPOULES

Ethylene oxide treatment seems a possible solution of this problem (see chapter 23) but Whittet (1956) has shown that traces of formaldehyde can enter cracked ampoules, and since ethylene oxide is much more penetrating its use cannot be recommended.

### 6 IN STERILITY TESTING

An ethylene oxide technique devised by Royce and Sykes (1955) greatly enhances the precision of sterility testing by reducing the chance of accidental contamination to a very low level.

Tests are performed in a gas-tight screen fitted with rubber gauntlets. The screen is metal but has windows, and part of the back can be removed for filling. Air can be admitted, or the internal atmosphere displaced, through sterile cotton-wool air filters.

The test samples, media and equipment for the transferences are introduced. Screw-capped containers of media are necessary because the high solubility of ethylene oxide could lead to inhibitory concentrations in tubes. Equipment is pre-sterilised by the usual heating methods so that the gas has to sterilise only the external surfaces. Sufficient chilled liquid ethylene oxide to produce an atmospheric concentration of 12.5 per cent v/v is put inside in a screw-capped bottle. This is opened and poured onto the floor after the screen has been tightly closed. The slight excess of pressure is allowed to escape through the filters which are then tightly closed. The aim is to sterilise with at least 10 per cent v/v (200 mg/litre), the excess provides more than adequate allowance for the initial escape and for absorption, e.g. in the gauntlets. After a 16 to 18 hr exposure overnight the gas is flushed out with sterile air and, before the tests are carried out, the gauntlets are left hanging outside the screen for an hour for desorption to take place.

*Advantages of this Technique are*

- (a) Greater reliability, because the chance of accidental contamination is extremely low. This is particularly valuable when the filtration method of testing is being used.
- (b) There is no need for scrubbing up, sterile clothing or an asepsis room.

*Disadvantages*

These are minor—

- (a) Movements are slightly restricted by the closed front of the screen and the gauntlets.
- (b) Nothing must be forgotten when the screen is loaded.

### ADVANTAGES OF ETHYLENE-OXIDE STERILISATION

- 1 It is suitable for thermolabile substances because it can be carried out at room temperature or only slightly above.
- 2 It does not damage moisture sensitive substances.

- and equipment because only a low humidity is required
- 3 Provided the container is made from one of the many materials that are permeable to the gas it can be used for pre-packed articles because of the great penetrating power of ethylene oxide
  - 4 Although ethylene oxide is a highly reactive compound comparatively few materials are damaged by the process
  - 5 It can be used in a closed screen to increase the reliability of sterility testing

#### DISADVANTAGES OF ETHYLENE OXIDE STERILISATION

- 1 It is slow Long exposures and desorption periods are necessary and, therefore, it is unsuitable in emergencies or for expensive equipment that must be frequently used

- 2 Although small batches of materials can be successfully sterilised with simple equipment, large batches require very expensive, elaborately instrumented sterilisers that need skilled and regular maintenance
- 3 The running costs are high, e.g. 4s /ft<sup>3</sup> of chamber space per cycle (Kelsey, 1961)
- 4 The hazards of inflammability, general toxicity and vesicant action necessitate special precautions

#### Conclusions

Ethylene oxide sterilisation is less reliable and more expensive than steam sterilisation and should never be used when the latter is practicable

Sterilisation by ionising radiations is more certain and may become more attractive economically for heat sensitive articles that are required in bulk, e.g. disposable catheters and syringes

### OTHER COMPOUNDS USED FOR GASEOUS STERILISATION

Like ethylene oxide these are alkylating agents

#### 1 FORMALDEHYDE

Nowadays the chief application of formaldehyde is the fumigation of empty rooms after infectious diseases (Extra Pharmacopoeia Supp., 1961). In the past it has been used, in special chambers, to sterilise catheters, syringes and other thermolabile hospital equipment, for these purposes it is inferior to ethylene oxide because—

- (a) *Its penetrating power is weak.* As a surface bactericide it is superior to ethylene oxide (concentrations of 3 to 10 mg/litre are adequate) but porous and wrapped materials are very difficult to sterilise. Some improvement results when a pre-vacuum is drawn and, particularly, if a high temperature is used, but the latter negates the value of the method for thermolabile articles.
- (b) *High concentrations are difficult to maintain in the atmosphere.* On contact with surfaces it deposits in the form of solid polymers or dissolves in moisture films.
- (c) *A high humidity is necessary.* This must be in excess of 60 per cent, and 75 per cent is often recommended.
- (d) *It is readily inactivated, e.g. by proteins and other organic matter.*
- (e) *Adsorbed gas is very difficult to remove.* Very long airing times are required.
- (f) *It is very irritating to the respiratory tract.*
- (g) *The pure substance cannot be kept at ordinary*

*temperatures.* Consequently, it is used either as Formaldehyde Solution (Formalin) B.P., which is an approximately 37 per cent w/w solution containing stabilisers to prevent deposition of solid polymers, or as tablets of one of its polymers, paraformaldehyde. The gas is vapourised from these sources by heating devices or, in the case of Formalin for fumigation, by the addition of potassium permanganate which produces heat by oxidation.

(For additional information see Public Health Lab Report, 1958, Abbott, Cockton and Jones, 1956, and Bullock and Rawlins 1954.)

#### 2 BETA PROPIOLACTONE

This relatively new compound resembles formaldehyde rather than ethylene oxide. It is highly bactericidal on exposed surfaces and concentrations of 5 mg/litre or less are used. Its penetrating power is small and a relative humidity of about 75 per cent is necessary. It is non-inflammable and has been used for fumigation and for the sterilisation of unwrapped instruments and closures. It damages nylon, polystyrene and polyvinyl chloride. It is a liquid at room temperature and vaporisation methods are necessary. Reports of carcinogenicity on mouse skin have restricted its development.

#### REFERENCES

- 1 ABBOTT, C. F., COCKTON, J. and JONES, W. (1956) Resistance of crystalline substances to gas sterilisation *J. Pharm. Pharmacol.* 8, 709-720

- 2 BRUCH, C W (1961) Gaseous sterilisation *Ann Rev Microbiol*, **15**, 245-262
- 3 BULLOCK, K. and RAWLINS, E A (1954) The bactericidal action of volatile substances. Part I Disinfection of powders containing spores by means of gaseous formaldehyde *J Pharm Pharmacol*, **6**, 859-876
- 4 EXTRA PHARMACOPEIA SUPP (1961a) *The Extra Pharmacopæia (Martindale) Supplement* The Pharmaceutical Press, London. 91-92
- 5 EXTRA PHARMACOPEIA SUPP (1961b) *ibid*. 91
- 6 FREEMAN, M A R and BARWELL, C F (1960) Ethylene oxide sterilisation in hospital practice *J Hyg, Camb*, **58**, 337-345
- 7 KELSEY, C (1961) Sterilisation by ethylene oxide *J clin Path*, **14**, 59-61
- 8 MAYR, G (1961) Equipment for ethylene oxide sterilisation *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London, 90-97
- 9 OPFELL, J B., HOHMANN, J P and LATHAM, A B (1959) Ethylene oxide sterilisation of spores in hygroscopic environments *J Amer pharm Ass, Sci Ed*, **48**, 617-619
- 10 PERKINS, J J and LLOYD, R S (1961) Applications and equipment for ethylene oxide sterilisation *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London, 76-90
- 11 PHILLIPS, C R (1961) The sterilising properties of ethylene oxide *ibid* 59-75
- 12 PUBLIC HEALTH LAB REPORT (1958) Report of the Committee on Formaldehyde Disinfection of the Public Health Laboratory Service *J Hyg, Camb*, **56**, 485-515
- 13 ROYCE, A (1959) Modern sterilising and aseptic techniques *Publ Pharm*, **16**, 235-241
- 14 ROYCE, A and BOWLER, C (1959) An indicator control device for ethylene oxide sterilisation *J Pharm Pharmacol*, **11**, 294T-298T
- 15 ROYCE, A and BOWLER, C (1961) Ethylene oxide sterilisation—some experiences and some practical limitations *J Pharm Pharmacol*, **13**, 87T-94T
- 16 ROYCE, A and SYKES, G (1955) A new approach to sterility testing *J Pharm Pharmacol*, **7**, 1046-1052
- 17 SUMMERS, F H (1962) Pharmacy in a plastic surgery hospital *M and B Pharmaceutical Bulletin*, **11**, 6-8
- 18 SUTARIA, R H and WILLIAMS, F H (1961) Ethylene oxide sterilisation in a hospital pharmacy *Pharm J*, **186**, 311-314
- 19 TATTERSALL, K (1961) Control of sterility in a manufacturing process *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London. 198-203
- 20 TESSLER, J (1961) Reaction of the sterilant ethylene oxide on plastics *Appl Microbiol*, **9**, 256
- 21 WHITTER, T D (1956) Sterilisation of ampoules, penetration of formaldehyde vapour *Pharm J*, **177**, 340
- 22 WILKINSON, G R (1960) Sterilisation of medical products 2 Gas sterilisation *Mfg Chem*, **31**, 479-483

## 28

## Sterilisation by Radiations

RADIATIONS can be divided into two groups—

- 1 *Electromagnetic waves*, e.g. infra-red radiation, ultra-violet light, X-rays and gamma rays
- 2 *Streams of minute particles of matter*, e.g. alpha and beta radiations

The various types of electromagnetic waves can be arranged in a continuous spectrum according to their wavelengths

Increasing wavelength

Radio waves	—centimetres or metres
Infra red radiation	—microns ( $10^{-3}$ mm)
Visible light	—Ångstrom units (Å) ( $10^{-7}$ mm)
Ultra-violet light	—do
X-rays	—X-units ( $10^{-10}$ mm)
Gamma rays	—do
Cosmic rays	

The units of measurement shown beside the radiations indicate the large differences in wavelength at different parts of the spectrum. For example, the approximate ranges of ultra-violet light, visible light and infra red radiation are 150 to 4,000 Å, 4,000 to 7,000 Å and 7,000 to 150,000 Å (0.7 to 15  $\mu$ ) respectively.

Infra-red radiation, ultra-violet light, gamma radiation and high velocity electrons (a type of beta radiation) are used for sterilisation. For reasons given later, X-, alpha and other types of beta radiation are unsuitable. Infra red radiation destroys micro-organisms by the heat produced when the rays are absorbed in the irradiated material. Electromagnetic radiations of shorter wavelength than visible light (e.g. ultra-violet light and gamma rays)

often directly damage molecules vital to living cells, particulate radiations behave similarly.

This chapter deals with sterilisation methods in which there is no significant rise in temperature. Infra-red sterilisation, which is really a special dry-heat technique, is described in connexion with syringes and instruments in the next chapter.

#### EXCITATION AND IONISATION

An atom consists of a small heavy nucleus surrounded by planetary electrons. As ultra-violet light, gamma rays and high-speed electrons pass through matter they give energy to the electrons of the constituent atoms, with one of two results. The electrons may acquire sufficient energy to tear themselves free from the atoms—this is called ionisation. Alternatively, the acquired energy may not be sufficient to permit electrons to escape but it raises the atoms to an excessively energetic state—this is known as excitation. Although excitation or ionisation of the atoms of essential molecules in micro-organisms can cause death, ionisation is far more effective.

Ultra-violet light is of relatively low energy and rarely causes ionisation—it destroys by excitation. Gamma rays and high velocity electrons are far more energetic and, therefore, produce ionisation; they are called ionising radiations and are much more efficient sterilising agents than ultra-violet light.

#### RADIATION ENERGIES

Although ultra-violet light and gamma radiation are wave motions and have no mass, they behave in contact with matter as if they consisted of small packets of energy, these are called quanta or photons.

The energies of electromagnetic and particulate radiations are expressed by the energies of their individual quanta or particles respectively. The

energy unit used is the electron volt (eV). A particle or quantum has an energy of 1 eV if it has the same kinetic energy as an electron accelerated through a potential difference of 1 volt. Although this unit is large enough for ultra-violet quanta it is inconveniently small for ionising radiations and the energies of the latter are given in millions of electron volts (MeV).

The quantum energy of the most effective ultra-

violet wavelength for sterilisation purposes is only about 5 eV. The quanta of the two important gamma rays emitted from  $^{60}\text{Co}$ , a radioactive isotope used in radiation sterilisation, have energies of 1.33 and 1.17 MeV. A typical energy for the high velocity electrons, used for the same purpose, is 4 MeV. Comparison of these figures explains the low ionising power of ultra-violet light.

### ULTRA-VIOLET LIGHT

Direct sunlight can destroy micro-organisms, which is largely due to ultra-violet rays of comparatively long wavelength. Shorter wavelengths that are far more damaging to living cells are emitted by the sun but, fortunately, are absorbed by the earth's atmosphere.

Only a narrow range of wavelengths is suitable for sterilisation because above 3,100 and below 2,000 Å antimicrobial activity is very low. This is illustrated by the figures in Table 28.1, from Summer (1953)—

Table 28.1

Wavelength ( $\text{\AA}$ )	Effectiveness (%)
2,200	25
2,537	97
2,650	100
3,000	10
3,200	0.4

Quantum energy increases as wavelength decreases and, therefore, the fall in efficiency below 2,650 Å indicates that the antibacterial activity must be influenced by another factor. In fact, there is considerable variation in the damage done to living cells by a fixed amount of ultra-violet energy at different wavelengths. This is because, to kill micro-organisms, the radiation must be absorbed by an essential cell component, and the degree to which this occurs at any particular wavelength depends on the chemical structure of the component. The wavelength of 2,650 Å and adjacent wavelengths are strongly absorbed by nucleoproteins, e.g. the deoxyribose nucleic acid protein of the cell nucleus, and disruption of these vital compounds would be expected to cause serious damage to an organism. Wavelengths absorbed by less essential substances are relatively harmless. These ideas are supported by the close relationship between the bactericidal efficiencies of different wavelengths and their degrees of absorption by nucleic acids. In addition, the more obvious effects of ultra-violet light on living

organisms, i.e. death, cessation of cell division and mutations, are probable results of damage to nucleoproteins.

All types of micro-organism can be destroyed by bactericidal wavelengths. Some mould spores have relatively high resistance but bacterial spores are only a few (2 to 10) times more resistant than vegetative bacteria. The different growth requirements of damaged cells, mentioned in connexion with sterility testing, have often been noticed in organisms treated with ultra-violet, some of which, although unable to divide and produce colonies in the dark can do so if exposed to light immediately after treatment (photoreactivation) or if certain metabolites are included in the medium (metabolic reactivation). There is some evidence for an indirect effect of ultra-violet light (cf. ionising radiations), this is believed due to hydrogen peroxide (a cell poison) produced by the action of the radiation on some of the ingredients of the medium in which the organisms are exposed.

#### Sources of Ultra-violet Light

The main method of generating ultra-violet light for sterilisation is by passing a low current at high voltage through mercury vapour in an evacuated glass tube. The planetary electrons of the outer orbit of the mercury atoms become excited and discharge their excess energy as ultra-violet radiation. About 90 per cent of this is at 2,537 Å which although not the wavelength of maximum efficiency, is highly microbicidal.

Because impurities, such as ferric iron, sulphur, iodine and titanium, in ordinary glass absorb antimicrobial wavelengths, particularly 2,537 Å (Stanworth, 1950), the tubes are made from quartz or Vycor (a quartz-like substance derived from borosilicate glass). Vycor has the advantage of absorbing the shorter ultra-violet rays (1,850–1,950 Å *cw*) that produce ozone from atmospheric oxygen. Ozone is antibacterial (Ingram and Barnes, 1954) but is harmful to human beings at concentrations considerably lower than those needed for bactericidal action.

also, because it is a very powerful oxidising agent, it attacks many materials, especially natural rubber. Quartz tubes, when used for applications where ozone could be harmful, e.g. the irradiation of occupied rooms, are jacketed with a liquid (e.g. brine) that suppresses the appropriate waveband. The popular size of tube for air treatment has an effective length of 2 ft but a half size is available for use in asepsis screens (for further information see the technical literature of Engelhart-Hanovia Ltd, Slough). Depreciation gradually takes place, and occasional measurements of ultra-violet intensity (see Medical Research Council Report, 1954 and Mellors, 1952) are desirable to ensure replacement before the bactericidal efficiency has fallen significantly. The life of a Vycor discharge tube (2 ft effective length) made by the above firm is about 4,000 hr., i.e. about 6 months, if used continuously.

The intensity of ultra-violet radiation is expressed as the energy received by a specified area, usually in microwatts/cm<sup>2</sup>. Intensities of from 1,000 to 6,000  $\mu\text{W}/\text{cm}^2$ , depending on the type of bacterium, will reduce populations of vegetative cells by about 90 per cent in 1 second. A surface 30 cm below a lamp might receive 500  $\mu\text{W}/\text{cm}^2$ , but the mean intensity in the air of an irradiated room is much lower, e.g. 20  $\mu\text{W}/\text{cm}^2$ . Equal products of time and intensity cause approximately the same degree of inactivation of micro-organisms and, therefore, correspondingly long exposure times can be used to compensate for low intensities. Air absorption of ultra-violet light is insignificant, and the decrease in intensity with distance from the source obeys the inverse square law approximately.

The output of low-current discharge lamps is affected by the surrounding temperature, this should not be less than 50°F and, preferably 20° to 30°F higher. Passage of air over the tubes causes cooling, and in situations such as the ducts of ventilating systems, where the air flow rate is considerable, a further increase of 10° to 15°F is desirable.

#### **Penetrating Power**

The most serious disadvantage of ultra-violet light as a sterilising agent is its poor penetrating power, this is a result of strong absorption by many substances. Therefore

1. Although it rapidly destroys naked or droplet-enclosed micro-organisms it is far less effective against organisms within or on the unexposed sides of dust particles or dried mucus. When used for the sterilisation of the air of asepsis rooms it should be combined with strict dust control measures and a

method, e.g. filtration or electrostatic precipitation, that removes atmospheric dust efficiently.

2. It is not applicable to the sterilisation of packed pharmaceuticals (cf ionising radiations).

3. Its use for the sterilisation of thermolabile medicaments and preparations, prior to aseptic packing, is severely limited. Although bactericidal wavelengths are not seriously absorbed by 3 to 4 cm of distilled water the presence of particles in suspension and organic compounds and certain inorganic salts (especially iron compounds) in solution markedly reduces transmission. To treat successfully preparations such as plasma and vaccines, that contain large amounts of organic matter, films only a fraction of a millimetre thick must be exposed. Vallet (1952) states that 75 per cent of the radiation at 2,537 Å is absorbed by an 0.2-mm film of plasma.

4. It is less effective against organisms in the atmosphere or on surfaces if the relative humidity is high, possibly because the cells are protected by radiation absorbing films of moisture that are absent at lower humidities. A relative humidity of less than 50 per cent appears desirable.

5. The radiation is partially screened by dust or grease on the lamp. Regular cleaning, preferably daily, is necessary. Usually a wipe with a clean damp cloth is sufficient, but if grease is tenacious and an organic solvent is used it must be one that leaves no residue (e.g. alcohol, but not I.M.S.)

#### **Hazards**

Bactericidal ultra-violet light causes acute conjunctivitis and erythema of the skin. Exposure of the eyes and skin to direct or high intensity reflected radiation must be prevented. Reflected light can be received from bright metal equipment or non-absorbing wall paint.

#### **Applications**

##### **I. IRRADIATION OF INCOMING AND/OR INTERNAL AIR OF THE STERILE FILLING AREAS OF ANTIBIOTIC PLANTS**

The incoming air may be exposed to batteries of discharge tubes installed in a section of the air duct immediately adjacent to the room. The duct is coated internally with aluminium paint to reflect the radiation and may contain thermostatically controlled heaters to provide a satisfactory working temperature for the tubes. The air velocity governs the residence time in the irradiated zone. Efficiency can be enhanced by humidity control and prefiltration to remove large dust particles.

Inside rooms the lamps are fitted near to the ceiling with the rays directed downwards. As

occupants receive direct radiation they must wear eyeshields (e.g. plastic face masks) and clothing that completely covers the skin (i.e. hoods, gowns with long sleeves and rubber gloves) (see Jones, 1952)

Ultra-violet radiation can provide air of adequate bacteriological quality for this purpose only if combined with a method for removing dust. The need to protect workers is a serious disadvantage.

## 2 AS AN AID TO ASEPSIS

- (a) *In manufacturing houses* Over the working area during the filling and closing of containers of sterile thermolabile products
- (b) *In hospitals* Inside a screen during aseptic processing

Ultra-violet light is helpful in these circumstances but pharmaceutical users, aware of the risk from dust-borne infection, are unlikely to relax full aseptic precautions and, therefore, the advantage may not be great.

## 3 TO PREVENT CROSS INFECTION IN HOSPITALS, SCHOOLS ETC

### (a) Indirect Method

Lamps are mounted above the heads of occupants, on the walls, or ceiling, and with the radiation directed upwards by suitable reflectors. The result is an irradiated zone across the top of the room through which the air is encouraged to pass by natural convection, radiators etc. Protection of occupants is unnecessary. The method is much less efficient than direct radiation but a bacteriological purity equivalent to sixty or more changes of air per hour is obtainable. (The effect of disinfection by ultra-violet irradiation on illness among school-

children is discussed in a Medical Research Council Report, 1954.)

### (b) Direct Method

Lamps are mounted over the doors of rooms and cubicles containing infectious patients to provide an intense bactericidal curtain down to the floor. This method is more widely used in America than in this country.

## 4 STERILISATION OF THERMOLABILE SUBSTANCES

Poor penetration, unreliability and the possibility of photochemical damage have largely prevented the application of this method to the sterilisation of thermolabile pharmaceuticals.

It has been used—

- (a) To destroy the virus of homologous serum jaundice in blood plasma

It is difficult to ensure adequate penetration and, therefore, complete destruction of the virus, without damage to plasma proteins.

- (b) To inactivate viruses and bacteria in vaccine production

Ultra-violet light has been successful in a few cases where heat and chemical treatment seriously affect antigenicity. In one method, to expose a sufficiently thin film, the suspension was run slowly down the inside of a rotating cylinder containing an axially fitted lamp (Collier, McClean and Vallet, 1955). A different technique is described by Hampton and Polson (1958).

(For further information on ultra-violet light as a sterilising agent see Bourdillon and Lidwell, 1948, Hollaender, 1955, and Lea, 1955.)

## IONISING RADIATIONS

Ionising energy can be obtained from machines or radioactive isotopes. Machine generated radiations include X rays, electrons, protons and alpha particles. Radioactive isotopes provide sources of alpha particles, beta particles and gamma rays. All ionising radiations, in adequate dosage, can destroy micro-organisms but most are unsuitable for the sterilisation of pharmaceutical and medical materials because they fail to satisfy one or more of the following requirements:

### Good Penetrating Power

X ray and gamma radiations penetrate well because they are uncharged, massless and have highly energetic quanta. For example, 10 per cent of the

original intensity of a beam of 1 MeV gamma rays remains after passage through 35 cm of water.

Alpha and beta particles have charge and mass and, consequently, their passage through matter is impeded by interaction with atomic fields and collisions with atomic particles. Alpha particles, which are doubly charged and heavy (they are the nuclei of helium atoms) have a range of only a few millimetres in water while beta particles (these have the mass of an electron and are negatively (electrons) or positively (positrons) charged), which have only a single charge and are much smaller (the mass ratio  $\alpha/\beta = 4/1,800$ ) can penetrate to about a centimetre. These penetrations are inadequate for routine sterilisation and, therefore, alpha- and beta particles

from isotope sources are not used. However, machine generated particles are more satisfactory because they have been accelerated to high velocities to increase their penetrating power.

### High Sterilising Efficiency

It seems that many organisms can be destroyed by a single ionisation in a radiation sensitive region (the target) of the cell (Lea 1955). Radiations, such as alpha particles and protons, that produce dense tracks of ions as they traverse matter cause far more ionisations than necessary in the target and, therefore are less efficient than radiations, such as electrons, X rays and gamma rays, with which ionisations are more sparsely distributed.

### Minimum Damage to Irradiated Materials

Exposure to ionising radiations can cause adverse chemical and physical changes. The risk is greater with particles that produce dense ionisations.

### Satisfactory Production Efficiency

X radiation suitable for sterilisation can be produced by bombarding a heavy metal target with a beam of high speed electrons but such a small proportion of the beam energy is converted to X rays that this type of radiation cannot compete economically with the alternatives.

The only radiations that adequately fulfil these four requirements are high speed electrons from machines and gamma rays from radioactive isotopes.

## 1 HIGH SPEED ELECTRONS

This type of sterilising radiation is most widely used in the United States of America. In a machine known as a Van de Graaff accelerator electrons are generated from a suitable source and then accelerated along a highly evacuated tube by a tremendous potential difference between the ends. The particles in the emergent beam are travelling at near to the speed of light and, depending on the accelerating voltage, have energies of from 1 to 4 MeV. The beam, which is narrow and intense is used to irradiate articles on a conveyor belt.

In England a machine (the travelling wave linear accelerator), in which a different method of acceleration is used has been developed. The electrons are passed into a horizontal tube along which an ultra short radio wave is travelling. This wave produces fields that are alternately positive and negative. Electrons ejected into a positive field are attracted, and therefore accelerated but electrons emitted into a negative field are repelled backwards. Consequently, the linear accelerator discharges electrons

in short bursts unlike the Van de Graaff accelerator which gives a steady stream. Each burst (pulse) is of about 2  $\mu$ sec duration. Machines producing 4 MeV electrons are being used for sterilisation studies.

One British pharmaceutical firm (Smith and Nephew Ltd) has installed a Van de Graaff accelerator.

(For further information see Trump, 1961 and Summer, 1952.)

## 2 GAMMA RAYS

Most gamma ray sterilisation in this country is carried out at the package irradiation plant at Wantage but Ethicon Ltd have a plant for the sterilisation of sutures. Radiation from the radioactive isotope of cobalt,  $^{60}\text{Co}$ , is used. Almost all of the unstable atoms of this isotope emit, when they disintegrate, two gamma rays in succession (in cascade). These have energies of 1.33 and 1.17 MeV, giving a total emission per disintegration of 2.5 MeV.  $^{60}\text{Co}$  has to be produced in an atomic pile its half life, which influences the frequency with which the source must be replenished and is the time for the activity to drop to half its initial value is 5.25 years.

It is possible that eventually  $^{60}\text{Co}$  will be replaced by the radioactive isotope of caesium  $^{137}\text{Cs}$ . This is a product of the disintegration of uranium and is a major constituent of the spent fuel rods from nuclear reactors. Because of its long half life, 30 years, it presents a formidable disposal problem that use for radiation sterilisation would solve. Its availability will increase with the growth of nuclear power stations. It emits a gamma ray of energy 0.66 MeV. At present it is used, together with the other disintegration products in the rods, in the Spent Fuel Rod Assembly at Harwell in which some commercial sterilisation is carried out.

The unit of radioactivity is the curie (c). This is the amount of any radioactive substance that undergoes  $3.7 \times 10^{10}$  disintegrations/second. At Wantage,  $^{60}\text{Co}$  sources of several hundred kilocuries (kc) are used, the isotope in the form of pellets is enclosed in double stainless steel tubes, 2 ft long by  $1\frac{1}{2}$  in in diameter, each containing 4,000 c. A row of these constitutes the source plaque. Articles are packed in boxes of standard size which are suspended from a monorail and sterilised by several slow passages around the plaque.

(For further information see Burnard 1961 and Powell, 1961.)

### Mode of Action

Ionising radiations cause both excitations and ionisations and their absorption is not affected by

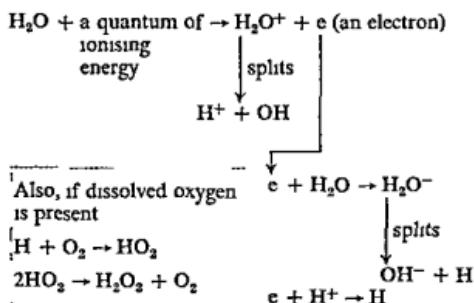
the structure of the molecules in the irradiated material (cf ultra-violet light) Harmful effects on micro-organisms and organic compounds may be the result of direct or indirect action

#### (a) DIRECTION ACTION (TARGET) THEORY

This proposes that every micro-organism and living cell contains a target region (or regions) so highly sensitive to radiation damage that even a single ionisation inside it causes inactivation or death. In very small organisms, such as bacteriophages and the small viruses, which appear to consist of a large central core of nucleic acid coated with a thin layer of protein, the targets are approximately the same size as the organisms themselves (see Lea, 1955) which suggests that nucleic acid is the sensitive material. In larger viruses and bacteria evidence favours a large number of smaller targets (probably radiation sensitive genes) ionisation in any one of which causes death.

#### (b) INDIRECT ACTION (CHEMICAL) THEORY

Absorption of radiation by the water, within or surrounding living cells, produces free radicals. These are powerful oxidising and reducing agents capable of damaging essential molecules and, therefore, causing death—



The oxidising radicals OH and HO<sub>2</sub>, and the hydrogen peroxide, are more destructive than the reducing radical, H.

#### Sterilising Dose

The dose unit is the *rad*—the amount of ionising radiation from any source that delivers 100 ergs of energy/G of absorbing material.

A report on the sterilisation of pharmaceuticals published by the Association of British Pharmaceutical Industry (A B P I Report, 1960) concludes that—

- (i) Gram negative organisms appear more sensitive than Gram positive organisms
- (ii) Spore producers are the most resistant,
- (iii) A dose of  $2.5 \times 10^6$  rads appears adequate to ensure sterility

Two of the least susceptible sporing bacteria are *Bacillus pumilis* and *Clostridium sporogenes*, the above dose reduces the numbers of these by factors of  $10^{10-12}$  and  $10^7$  respectively. *Bacillus pumilis* is often used to test radiation sterilisation processes.

#### Sterilisation Time

The intense beams or pulses of electrons from accelerators can deliver a sterilisation dose in a fraction of a second to a few seconds, depending on the size and density of the material or article being irradiated. With isotope sources, because of the diffuse and penetrating nature of their emissions, the dose rate is much less and, therefore, the sterilising dose has to be accumulated over several days (e.g. 3 to  $3\frac{1}{2}$ ). However, as partial compensation for this, the volume of material that can be exposed at the same time is much larger than with accelerators.

#### Penetration

This is not often a difficulty with isotope sources because of the great penetrating power of gamma radiation. Unlike electron beams, gamma radiation does not have a finite range in matter, its intensity decreases exponentially with the thickness of the absorber.

The main problems are—

- (i) To ensure even and adequate dosage throughout the exposed articles. This is overcome by packing the sterilising boxes to a specified density and exposing opposite faces on the two sides of the source (Fig. 28 1).
- (ii) To utilise some of the radiation that passes right through the articles. This is achieved by exposing several rows of boxes on each side of the source and moving each box at intervals so that it spends several minutes at each position in every row (Fig. 28 1).
- (iii) To waste as little as possible of the omnidirectional radiation from the source. This is partially solved by having a tier of boxes at each position (Fig. 28 1). Every box makes a circuit at each level. (For full details see Burnard, 1961.)

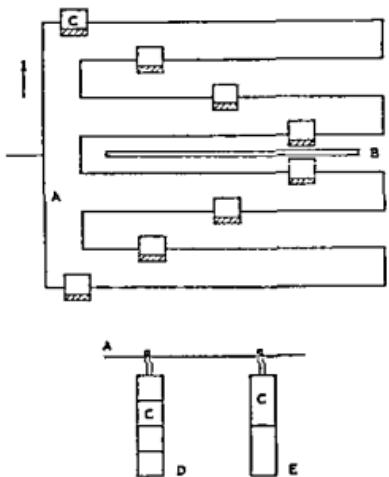


Fig. 281 MONORAIL SYSTEM FOR STERILISATION BY GAMMA RADIATION

A—monorail  
B—source plaque  
C—packages (shown at only a few positions on the rail)—one side is shaded to illustrate exposure of opposite faces on the two sides of the source  
D & E—tiers of 4 and 2 packages respectively

The range of machine-produced electrons depends on the accelerating voltage. The maximum penetration is approximately 1 cm in unit density material for every 2 MeV of electron energy. Maximum ionisation is at about one-third of this distance because many of the electrons are scattered by collisions with atomic particles in the absorber and the resulting deflections are at wide angles to the original path. Ionisation is not maximal at the incident surface because a charged particle produces more ionisations towards the end of its path. An effective dose is received up to about 60 per cent of the maximum range. Greater thicknesses of material can be given a sterilising dose by—

- Irradiation from both sides, using two beams
- Turning the article over between two passages under the beam
- Rotating the article during one traverse

The last two methods are not suitable for materials that move during turning and rotation, e.g. liquids and loosely packed powders. These techniques increase the thickness receiving an effective dose by

rather more than twice because of the summation of the ionisations produced beyond the effective dose range by the two beams or exposures.

Penetration also depends on the density of the irradiated material. It can be approximately calculated by dividing the penetration into unit density material by the density of the material in question. Summer (1952) gives the following figures for the densities of packaging materials: aluminium 2.7, glass 2.4 to 3 and plastics 1.1 to 1.6. Where possible, low density containers, e.g. plastics, are used.

Penetration can be studied by exposing containers filled with an agar gel containing a radiation indicator. A popular indicator system contains chloroform and methyl yellow; chlorine produced from the former by the radiation converts the methyl yellow to a red dye and the intensity of the red colour gives an indication of the amount of energy absorbed. This reaction is also the basis of a radiosensitive paint that when coated on to labels indicates that a sterilising dose has been received at the container surface (Report, 1963).

#### Deleterious Effects

Radiation sterilisation appears an attractive method for thermolabile medicaments and equipment because the rise in temperature caused by a sterilising dose is very small—about 4°C. However, the A B P I investigation of the effects of radiation on pharmaceutical products (A B P I Report, 1960) led to the conclusion that in many instances  $2.5 \times 10^6$  rad produces changes that may make the preparation unacceptable for administration or presentation.

Undesirable effects include decomposition, immediate or after storage, and alterations in colour, texture and solubility. Potency changes range from almost nil or nil (e.g. certain antibiotics and steroid hormones) to serious loss (insulin, posterior pituitary hormones and cyanocobalamin). Alterations in colour are common and although sometimes there is no associated loss of activity the preparation is less acceptable for sale. Because of the indirect effect of radiation destruction is often greater when substances are irradiated in solution (e.g. heparin).

Plasma can be sterilised by gamma radiation but the red cells of whole blood fragment. Rancidification of fats and development of unpleasant flavours in foodstuffs occur frequently.

Ordinary types of clear glass become brown. Special glasses that are unaffected have been developed but are expensive. Occasionally, pieces of normal glass are included within packages as indicators of radiation treatment.

## Reduction of Undesirable Effects

Several methods of controlling unwanted radiation damage have been investigated, particularly in connexion with the sterilisation of foods (D S I R Report, 1952)

### 1 Irradiation in the Frozen State

This is practicable when electrons are used because the exposure is so short. Freezing slows down the diffusion of free radicals and, therefore, fewer reach the molecules of sensitive substances.

### 2 Addition of Chemical Protectants

A substance, e.g. ascorbic acid, cysteine, thiourea, is added to compete for the free radicals. Sulphydryl compounds were investigated because some, e.g. cysteine, were known to protect healthy cells of the human body from damage by X rays.

### 3 Removal of Oxygen

This decreases oxidative effects possibly by causing a reduction in the number of oxidising free radicals.

### 4 Use of Pulsed Radiation

i.e. the discontinuous electron beam generated by the linear accelerator.

Evidence that a 1  $\mu$ sec exposure to high velocity electrons is insufficient to initiate chemical reactions but sufficient to kill micro-organisms led to the suggestion that the use of very short pulsed radiation might prevent chemical damage without affecting sterilisation efficiency.

The future of these techniques in the sterilisation of pharmaceuticals is at present uncertain.

## Applications

Although radiation sterilisation of medicaments may not develop quickly, because of the need to prove the absence of short- and long term deleterious effects, the method is firmly established for disposable surgical materials and equipment. Articles regularly treated on a commercial scale include plastic syringes (Hunter, 1961) and catheters (Darnady *et al.*, 1961), hypodermic needles and scalpel blades, adhesive dressings (Dow, 1961) and catgut. Popular packaging materials include plastic films and aluminium foil.

## ADVANTAGES

- 1 The temperature rise is insignificant
- 2 The processes can be continuous because exposure

is so short (machines) or a large amount of material can be treated at once (isotopes).

- 3 There is no aseptic handling since sterilisation can be performed after packing in the final containers.
- 4 The methods are reliable and can be very accurately controlled.
- 5 Dry, moist and, with electrons, frozen materials can be treated.
- 6 Some bacterial and viral vaccines can be sterilised without loss of antigenicity.

## DISADVANTAGES

- 1 Capital and replacement costs are high. Preferably, isotopes should be used 24 hours a day because the radiation cannot be switched off.
- 2 Elaborate and expensive precautions must be taken to protect operators from the harmful effects of ionising radiations.

For isotopes, the radiation chamber is surrounded with concrete several feet thick, the door is stepped to prevent escape of radiation through the surrounding cracks, and automatic controls are installed, e.g. to prevent raising of the source while the door is open, and vice versa. When not in use the source is lowered into a deep water-filled pond in a concrete pit.

The problems are smaller with machines because they can be switched off when out of use and the electrons are less penetrating than gamma rays. However, a penetrating form of X ray known as bremsstrahlung is produced by the stopping of electrons by matter and, since this escapes in all directions considerable shielding must be provided.

- 3 Deleterious changes are produced in many medicaments, fats and foods. Nevertheless, at the dose levels normally used there is no danger of residual radioactivity in irradiated products.

(For further information see Hollaender, 1954 and Horne, 1956.)

## REFERENCES

- 1 A B P I REPORT (1960) *Report of a working party established by the Association of British Pharmaceutical Industry and others on the use of gamma radiation sources for the sterilisation of pharmaceutical products*. Association of British Pharmaceutical Industry, Tavistock Square, W C 1
- 2 BOURDILLON, R B and LIDWELL O M (1948) Air disinfection by ultra violet radiation. Survey of chief factors concerned. *Medical Research Council Special Report Series No 262 Studies*

- in Air Hygiene* Her Majesty's Stationery Office, London. 173-176
- 3 BURNARD, L. G (1961) Design and production of irradiation plants *Symposium on the sterilisation of surgical dressings* The Pharmaceutical Press, London, 34-45
- 4 COLLIER, L. H., MCCLEAN, D and VALLET, L (1955) The antigenicity of ultra violet irradiated vaccinia virus *J Hyg, Camb.*, 53, 513-534
- 5 DARMADY, E. M., HUGHES, K. E. A., BURT, M. M., FREEMAN, B. M. and POWELL, D. B. (1961) Radiation sterilisation. *J clin Path.*, 14, 55-58
- 6 DOW, J (1961) Sterilisation of adhesive dressings *Symposium on the sterilisation of surgical dressings* The Pharmaceutical Press, London, 29-31
- 7 D.S.I.R. REPORT (1952) Report of the Department of Scientific and Industrial Research on Drug sterilisation by electrons and atomic rays *Mfg Chem.*, 23, 408-410
- 8 HAMPTON, J. W. F. and POLSON, A. (1958) Studies on poliomyelitis virus III. The use of ultraviolet light as an additional means of inactivation of formalinised vaccine *J Hyg, Camb.*, 56, 266-270
- 9 HOMAENDER, A. (1954) Editor *Radiation biology Vol I High energy radiations* McGraw Hill, New York.
- 10 HOMAENDER, A. (1955) Editor *Radiation biology Vol II Ultra violet light and related radiations* McGraw Hill, New York.
- 11 HORNE, T (1956) Sterilisation by radiation *Pharm J.*, 176, 27-29
- 12 HUNTER, C. L. F. (1961) Sterilisation of disposable hypodermic syringes *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London, 32-33
- 13 INGRAM, M. and BARNES, E. (1954) Sterilisation by means of ozone. *J appl Bact.*, 17, 246-271
- 14 JONES, W (1952) Aseptic conditions for the filling of penicillin. *Pharm J.*, 168, 213-216, 234-236
- 15 LEA, D. E. (1955) *Actions of radiations on living cells* 2nd. Ed. University Press, Cambridge
- 16 MEDICAL RESEARCH COUNCIL REPORT (1954) *Medical Research Council Special Report No 283 Air disinfection with ultra violet irradiation*. Her Majesty's Stationery Office, London.
- 17 MELLORS, H. (1952) Ultra violet irradiation of air supplies *J appl Chem.*, 2, 68-70
- 18 POWELL, D. B. (1961) Application of radiation sterilisation to surgical materials *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London. 9-16
- 19 REPORT (1963) Sterilisation by irradiation. *Pharm J.*, 190, 79
- 20 STANWORTH, J. E. (1950) Transmission of bactericidal radiation through glass *Nature, Lond.*, 165, 724-725
- 21 SUMMER, W (1952) Cold sterilisation. *Mfg Chem.*, 23, 451-455
- 22 SUMMER, W (1953) Odour control with ultraviolet radiation. *Mfg Chem.*, 24, 105-110
- 23 TRUMP, J. G (1961) High energy electrons for the sterilisation of surgical materials *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London. 16-28
- 24 VALLET, L. (1952) Sterilisation by ultraviolet radiation *Chem and Drug.*, 157, 845-847

## Sterilisation of Equipment and Other Articles

### SYRINGES

The care taken in the preparation of parenteral solutions is negated if the syringes used in their administration are not cleaned and sterilised by efficient methods. Since the number used daily in a hospital or hospital group is large, the establishment of a separate unit for syringe processing is desirable and practicable. The planning and organisation of central syringe services have been discussed in a Nuffield Report (1957), many hospital pharmacists have described the units in their hospitals (e.g. Grainger, 1956; Hadgraft, 1959; Roberts, 1957) and details of an industrial service have been given by Harris (1961). Authoritative recommendations on the sterilisation, use and care of syringes will be found in M.R.C. Memorandum No. 41 (1962).

#### A. Non-disposable Syringes

##### 1 ALL-GLASS (Fig. 29 1a)

###### *Advantages*

- (a) This type is relatively easy to clean
- (b) Since all parts are transparent or translucent, unremoved dirt is readily detected
- (c) Sterilisation is relatively uncomplicated

###### *Disadvantages*

The nozzle is fragile.

##### 2 HALF-RECORD (Fig. 29 1b, c)

These differ from the all glass type in having a metal needle mount. This may be cemented to the barrel (b) or sweated on to a glass boss at its end (c).

###### *Advantages*

The nozzle is strong, particularly in type b.

###### *Disadvantages*

- (a) They are more difficult to clean thoroughly. In type c it is difficult to eliminate, during manu-

facture, a small ledge of glass inside the nozzle, and this collects dirt.

- (b) Absolute cleanliness within the nozzle cannot be confirmed.
- (c) Vintage syringes of type b will not withstand dry heat sterilisation. The parts may separate because of the low melting point of the cementing alloy or the barrel may fracture because the cement is insufficiently plastic to buffer the unequal expansion rates of glass and metal. Modern cements are free from these faults and can be safely heated to about 200°C.
- (d) In time, the metal parts may corrode.

Half Record, like all glass syringes, have glass plungers. If these fit badly the syringe is difficult or impossible to use. Barrels must always be fitted with their correct plungers (indicated by numbers marked on the glass); alternatively, interchangeable syringes can be used. The latter are costly but this is largely

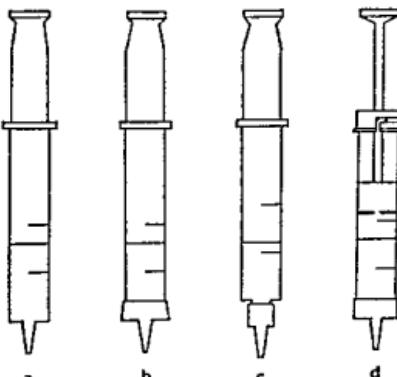


Fig. 29 1 GLASS AND GLASS METAL SYRINGES

balanced by other factors such as elimination of sorting time and easy replacement of broken parts

### 3 RECORD (Fig 29.1d)

This has a glass barrel with metal mounts top and bottom and a plunger that is completely or part metal.

#### *Advantages*

- (a) Durability This is still a popular type in general medical practice
- (b) The plunger usually fits well and does not easily seize, around its centre is a split ring that acts as a brake when the syringe is inverted

#### *Disadvantages*

- (a) It is the most difficult type to clean because of the many crevices
- (b) Metal-ended plungers must be separated from the barrels before sterilisation because expansion of the metal breaks the glass. Syringes with ceramic-ended plungers can be sterilised assembled.

Disadvantages (c) and (d) of the half-Record type also apply

### 4 NYLON

These are of similar design to an all glass syringe except that the plunger carries, near to its end, a rubber washer to prevent leakage (Fig. 29.2)

#### *Advantages*

- (a) Light in weight.
- (b) Unbreakable This is particularly valuable when breakage would create a hazard, e.g. for injections of radioactive isotopes

#### *Disadvantages*

- (a) The presence of the washer complicates—
  - (i) Cleaning—because it must be removed to clean the underlying groove



Fig. 29.2 TIP OF NYLON PLUNGER SHOWING WASHER

(ii) Sterilisation—because it is damaged by dry heat, autoclaving is recommended

- (b) They discolour with repeated use
- (c) Plunger movement is not very smooth and worsens with age.
- (d) Air bubbles accidentally drawn into the barrel often adhere tenaciously to the plastic and make accurate measurement impossible
- (e) Nylon reacts with certain bactericides and other chemicals (see chapter 21)  
(See also Richards and Whittet, 1958)

Ease of cleaning is of overriding importance because residues decrease the efficiency of sterilisation processes and may adversely affect the ingredients of the injection when the syringe is used. Consequently, all glass types are preferable, they are used in many services, but occasionally the type b half-Record is chosen because of its more robust nozzle

### B Disposable Syringes

Disposable syringes, intended for use on one occasion only, are most often made from transparent polystyrene, sometimes the plunger is of a white opaque variety of the same material. At the end of the plunger is a rubber grummet that may have been treated with a silicone (Fig. 29.3). They are obtained from commercial sources and, generally, are sterilised by gamma radiation. Eventually they may become cheap enough to replace non-disposable types for many purposes.

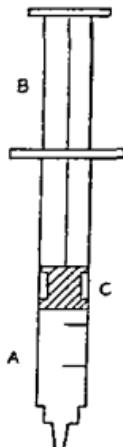


Fig. 29.3 DISPOSABLE PLASTIC SYRINGE

A—transparent polystyrene barrel  
B—opaque polystyrene plunger  
C—rubber grummet

### General Features of Syringes

Syringes with two types of nozzle, Record and Luer, are used. The latter has a less steep taper and, therefore, the needle grips it more tightly, it is recommended in the British Standard for Hypodermic Syringes (B S 1263 1946) and is fast replacing the Record type. Needles with the corresponding hub must be provided.

Glass parts should be neutral or borosilicate (chapter 21) and, ideally, the barrels should be unground since this facilitates observation of the contents. Calibrations must be irremovable, e.g. kilned into the glass.

The commonest sizes for general purposes are 2, 5, 10 and 20 ml. Specially calibrated syringes are available for Insulin (B S 1619 1962) and the tuberculinus.

### Needles

#### NON DISPOSABLE

These have a stainless steel shank and a nickel plated brass hub. They are numbered according to the gauge and length of the tubing used for the former. Popular sizes are Hypodermic 1 (often supplied with 5, 10 and 20 ml syringes), 12 and 14 (for suspensions) and 15 to 18 (for aqueous solutions).

#### DISPOSABLE

These have a certain future because of the advantages from always using a new sharp needle and the elimination of the tedious, time-consuming and costly cleaning and sharpening processes. They are sterilised by gamma radiation or ethylene oxide gas.

Each consists of a stainless steel shank in a plastic hub and several ingenious methods of packaging have been designed to provide good physical and bacteriological protection and facilitate aseptic attachment to the syringe.

### Cleaning Syringes

An efficient cleaning process for syringes is as important as for injection containers. It is complicated by the invisibility and relative inaccessibility of some of the parts, e.g. barrel nozzles and needle shanks. The following are essential stages, details are influenced by personal experience and the batch size.

#### 1 IMMEDIATE RINSING AFTER USE

All syringe users must be made fully aware of the importance of this stage. Syringes carrying dried deposits of materials such as blood, proteins and antibiotic suspensions may, at best, take many times longer to process than immediately and well rinsed syringes and, at worst, be impossible to clean com-

pletely. Rinsing until there is no visible residue is desirable and can be encouraged by issuing to users a suitable solution. Grainger (1956) recommends—

Green soft soap	25% w/v
Methylated spirit	25% v/v
Sodium hexametaphosphate	4% w/v
Glycerin	12% w/v
Water	to 100

This is diluted 1 to 4 for use.

#### 2 SOAKING

On arrival in the unit the barrels and plungers are separated, if possible. Those that are stuck are not released until after soaking. An hour in hot detergent solution is common practice. Some authorities object to organic detergents because of the difficulty of complete removal afterwards and the risks of syringe seizure and contamination of injections from dried films. Others have experienced no difficulty. Generally non-ionic types are preferred. Alternatives include liquid soap (1 in 10) and Steroxol (Laporte Chemicals Ltd)—a solution containing sodium hypochlorite (which helps to destroy deposits by oxidation) and sodium hexametaphosphate.

#### 3 RELEASE OF STUCK PLUNGERS

Plungers that soaking has not loosened are released with a syringe releaser (e.g. see Matthews, 1957).

#### 4 CLEANING

##### (a) By Ultrasonics

Ultrasonics are high energy sound waves of frequencies that are entirely or almost beyond the range of human hearing. The average person cannot detect sound of frequency greater than 14 kilocycles per second (kc/s).

Ultrasonics cause violent agitation when transmitted through a liquid. This is the result of the explosive formation and collapse of gas and vapour-filled cavities (cavitation). The resulting pressures are very high and rapidly dislodge dirt from articles immersed in the liquid, thus greatly improving the efficiency of cleaning solutions.

Ultrasonic cleaning equipment has two major parts, a generator to provide powerful pulses of electrical energy (e.g. at 40 kc/s) and a transducer to convert these pulses into mechanical vibrations of the same frequency. The transducer is coupled to the tank of cleaning fluid.

Two types of transducer are suitable for ultrasonic cleaning. Their underlying principles are—

(i) *Piezo-electric Type*. If two opposite parallel faces of a rectangular crystal of certain substances (e.g.

barium titanate) are brought to different potentials the crystal expands or contracts according to the direction of the current. The use of high-frequency alternating current produces vibrations in the ultrasonic range.

- (ii) *Magneto strictive Type* If a rod-shaped nickel laminate (or a similar ferro-magnetic material) is placed inside a solenoid through which alternating current is passing it vibrates in its longitudinal axis. Ultrasonic vibrations are produced if the current frequency is in the correct range.

Syringe cleaning equipment based on the piezoelectric principle has been described by Bradley, Morton and Frame (1959), and the successful use, for instrument cleaning, of an apparatus employing the magneto strictive effect has been reported by Myers and Goodman (1960).

For syringes that are not grossly soiled a treatment of about 5 minutes in a weak detergent solution at 60°C is adequate. Higher temperatures damage crystal transducers but can be used with magnetostriuctive types. Tenacious deposits may require special procedures (see Bradley *et al.*)

The method is most valuable for the insides of syringe nozzles because these are difficult to clean by traditional techniques. Since ceramic markings are removed and metal glass unions may be loosened, syringes with these features should not be cleaned in this way.

#### *(b) By High pressure Jets*

Jets of hot cleaning fluids are forced under pressure through the barrels. This method is used either alone or, to remove the last traces of loosened debris, after ultrasonic cleaning.

#### *(c) By Rotating Brushes*

Nylon brushes in a variety of sizes and with special nozzle-cleaning tufts at their tips are used in specially designed machines in which they can be rotated at about 1,000 rev/min. Generally, they are combined with one or both of the previous methods. There is a risk of scratching particularly from the tip of a worn brush.

#### **5 RINSING**

Complete removal of cleaning fluids, debris and detached bristles is achieved by several rinses first in hot tap water and finally in freshly-distilled water.

#### **6 DRYING**

The parts are separately dried in a drying oven and afterwards inspected, those with deposits or defects are recleaned or discarded respectively.

### Processing of Needles

#### **1 IMMEDIATE TREATMENT**

The user has two responsibilities—to clean the needle immediately after use and to protect its tip. After use for suspensions and sticky materials, cleaning should include passage of a stilette. The tip can be protected by plunging it into a thick pad of foamed plastic; similar pads are valuable at several stages of processing.

#### **2 SOAKING AND CLEANING**

In the unit the needles are first soaked in a cleaning fluid (for example, see Grainger (1956) and Harris, 1961). Then, if necessary the lumens are cleared with a stilette and the hubs are cleaned with fabric on a swab stick, a pipe cleaner or a tiny rotating brush. Afterwards, they may be attached to a suitable manifold and flushed under pressure with hot detergent, rinse waters and, to dry, I M S or air. Ultrasonic cleaning is not very successful except for the hubs; it is difficult to get fluid into the lumens and in its absence cavitation and, therefore, cleaning cannot take place.

#### **3 SHARPENING**

The tips are checked under magnification and, if necessary, are resharpened. A motorised fine stone and/or an Arkansas slipstone are used (M.R.C. War Memorandum No. 15, 1945). Sharpness is confirmed by inspection or by the absence of a characteristic 'ping' when the tip is passed through a piece of rubber glove stretched over a jar. Residual particles of metal are removed with a stilette and high pressure washing; the needles are then dried. Any showing signs of corrosion (the needle hub junction is the most likely place) are discarded.

The difficulty of confirming absolute cleanliness in a processed needle is another factor in favour of disposable types.

#### **Lubrication**

In most, but not all, services syringes are lubricated to give smoother action and better compression. Liquid paraffin, alone or dissolved in ether or petroleum ether, has been widely used but is no longer recommended because of the risk of injecting traces of oil; also, it often causes discolouration of the syringe, which can lead to the plunger sticking. Silicones are more satisfactory; they do not discolour the glass or, if applied carefully, contaminate the contents of the syringe. Occasionally, silicones that require baking to the glass surface are used (see chapter 21) and then only occasional replacement is

necessary. More often, types that remain fluid during sterilisation are preferred but they must be reapplied after each cleaning process, these include MS (Midland Silicone) 550, MS 510/500 and Repelcote, all obtainable from Hopkins and Williams, Chadwell Heath. Repelcote is supplied ready-diluted with an organic solvent, the others are used neat or in dilutions from 10 to 50 per cent in ether, petroleum ether or carbon tetrachloride. Ether and petroleum ether are highly inflammable and there is evidence (Fowler, 1953) that dry-heat sterilisation converts residual traces of the former to acetic acid, which attacks parts of the hub where plating has been worn away and produces drops of copper acetate.

The lubricant is applied to the plunger either with a sponge or by dipping in the liquid to about one-third of its length. Then it is spread evenly by working the plunger in the barrel. Lubrication should be done in a very clean atmosphere because traces of dust and hair can cause seizing.

#### Assembly

An ideal syringe container should—

- 1 Give good physical and bacteriological protection to the contents
- 2 Have an efficient seal that is easily applied and removed
- 3 Facilitate sterilisation
- 4 Permit aseptic removal of the contents
- 5 Be easy to clean thoroughly
- 6 Be transparent

#### TYPES OF CONTAINER

##### (a) Cellulose Film Envelopes

Each syringe is packed in an envelope of appropriate size. The needle may be attached or supplied separately in the same or a different envelope. The needle tip is protected by covering the shank with a length of transparent drinking straw. The envelopes are sealed with cement or cellulose tape.

#### Advantages

- 1 Transparency
- 2 Lightness in weight
- 3 A new and, therefore, clean envelope is used each time

#### Disadvantages

- 1 Poor physical protection of the contents
- 2 They become brittle at slightly above the temperature normally used for hot air sterilisation and although this disappears as the film reabsorbs atmospheric moisture prior handling may produce

tiny cracks and holes through which contaminants can pass

- 3 The method is costly, because each envelope is used once only

Nylon film has similar disadvantages

The M R C Memorandum does not recommend envelopes

##### (b) Glass Test tubes

The syringe is packed with the flange of its barrel resting on the rim of the tube. The projecting parts are covered with Kraft paper or cellulose film and sealed with a special tape.

If the needle is attached it is advisable for it to hang into a piece of drinking straw or annealed glass tubing (Fig. 29 4) to protect the tip if the needle works loose during sterilisation or handling, this is more likely with all glass syringes because of the different expansion rates of glass and metal.

#### Advantages

- 1 Transparency
- 2 Good physical protection of the contents
- 3 Ease of cleaning and inspection for cleanliness afterwards

#### Disadvantages

- 1 They are breakable. This is a hazard to workers in the service. Borosilicate tubes are desirable but expensive.



Fig. 29 4

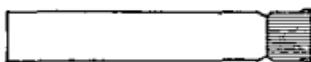


Fig 29.5 ALUMINIUM TUBE WITH FOIL CAP

- 2 The tape makes opening rather fiddling
- 3 The paper cover has the first two disadvantages of cellulose envelopes

To overcome disadvantages 2 and 3 special shaped tubes, in which the syringe is entirely enclosed and which can be sealed with aluminium foil caps, have been invented (e.g. White, 1950) but costs limit their use. Alternatively, special springs can be bought for placing at the bottom of ordinary tubes to allow the syringe to be completely inside without excessive movement or the needle tip touching the base (Cook, 1958), this method is inexpensive.

#### (c) Aluminium Tubes

These can be of spun or extruded aluminium. The latter are more popular because they are cheaper, lighter and heat up more quickly (Fig. 29.5). The most popular spun type (Davis, 1952) has a permanent cap but extruded types are sealed with foil.

#### Advantages

- 1 Good physical and bacteriological protection of the contents
- 2 Long life
- 3 The seals are easily applied and are impermeable to dust and usually to bacteria

#### Disadvantages

- 1 The contents are invisible
- 2 Cleaning and checking for cleanliness are more difficult than with glass tubes
- 3 Occasionally aluminium foil caps contain pin holes large enough for contaminants to enter. Additional protection can be given by plugging the tube before sealing but a covering of muslin is desirable to prevent contamination of the needle tip, or seizing of the plunger by loose fibres
- 4 Foil-capped tubes cannot be autoclaved because the steam cannot penetrate to the syringe. The spun tube, recommended by Davis, can be sterilised by moist or dry heat, as Fig. 29.6 shows

#### (d) Needle Tubes

When needles are supplied separately they are usually packed in glass tubes. One method is shown in Fig. 24.21 but in syringe services waisted (hour-glass) tubes are more common (Fig. 29.7). They are closed

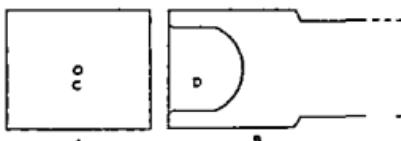


Fig 29.6 TOP OF SPUN-ALUMINIUM TUBE

When cap A is placed on neck B with the hole C over the cut-away part D the container may be autoclaved, because steam can enter. For dry heat sterilisation, and immediately after autoclaving the hole is turned away from D.

with aluminium foil caps (Hadgraft, 1959, Raine, 1958)

#### (e) Multi packs

The inclusion of several syringes and/or needles in one container is a hazardous method of packaging. To give adequate physical protection, tins are necessary and, as explained in chapter 22, these inhibit sterilisation because the lids fit tightly and large air pockets become trapped inside. The frequent opening of the pack during use progressively increases the risk of contaminating the remainder and used syringes may be accidentally replaced.

#### GENERAL PROCEDURE

Preferably, syringes should be sterilised with their needles attached because careless assembly before use could cause contamination. However, this is not always practicable since, to cover the different requirements of various users, it may be necessary to supply a range of needle sizes, which can be done more economically by keeping the needles separate.

When needles are attached they are fitted in such a way that when they are fully tightened their bevels are in line with the calibrations on the barrel. This ensures that the latter are clearly visible when the needle is correctly orientated for injection.

The items are tubed appropriately and, if desired, plugged with covered non-absorbent cotton wool. The foil caps are put on and tightened by insertion into the orifice of a bench machine that applies pressure all round.

In some services, after sterilisation, a metal shitter or a piece of thread is placed over the cap and held in position with a plastic ring. The latter distinguishes a sterilised syringe and, if coloured, can indicate the type of content. (Coloured foil caps are

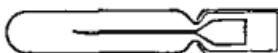


Fig 29.7 WAISTED NEEDLE TUBE

also available) The slitter or thread facilitates removal of the ring, this method is not used for opening the inner foil cap because the presence of the slitter reduces the efficiency of the seal. Some foil caps have tabs to simplify tearing.

### Sterilisation

The only pharmaceutically-acceptable methods for sterilising non-disposable syringes are dry heat and steam under pressure. For many years, boiling and storage in alcohol have been popular in general medical practice and, to a smaller extent, in hospitals, but they are unreliable (see M.R.C. Memorandum No. 41, 1962 and M.R.C. War Memorandum No. 15, 1945) and will not be considered further.

#### 1 STEAM UNDER PRESSURE

This is not the most satisfactory way of sterilising a syringe because of the difficulty of ensuring steam contact with every surface. When used—

(a) The closure of the container must be steam permeable, pressed foil caps are not. A cotton-wool plug with a paper cover is suitable.

(b) Dry syringes or syringes lubricated with fluid silicones or paraffin must not be sterilised assembled because the steam cannot reach the adjacent surfaces of the barrels and plungers, particularly when these are coated with a water repellent material. If no lubricant has been used or the surface has been siliconed by the baking process disassembly is unnecessary if the adjacent surfaces are made wet.

(c) The containers should be sterilised on their sides to encourage air drainage (see chapter 22).

#### 2 DRY HEAT

##### (a) Hot-air Oven

This is one of the most suitable methods if the apparatus is of good design and used and controlled correctly (see chapter 22).

The M.R.C. Memorandum recommends an exposure of 160°C for 1 hr.

##### (b) Infra-red Conveyor Ovens

Infra-red radiation covers a band of electromagnetic wavelengths beyond the red end of the visible spectrum (see chapter 28). It is a thermal radiation, i.e. when absorbed its energy is converted to heat and, therefore, it is often known simply as radiant heat. The importance of radiant heating in a hot-air oven was indicated in chapter 22. The infra red conveyor oven makes maximum use of this highly efficient means of heat transfer.

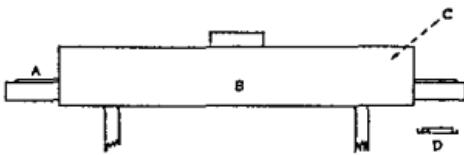


Fig. 29.8 INFRA-RED CONVEYOR OVEN

A—conveyor belt C—position of elements  
B—insulated tunnel D—tray of syringes to same scale

The advantages of radiation over convection and conduction as a means of heat transfer include—

- The heat is conveyed instantly from the source to the load. The velocity of all electromagnetic radiations is 186,000 miles/sec.
- The rate of energy transfer is constant because, unlike convection and conduction, it is unaffected by the temperature differential between the source and the load.
- It is virtually unaffected by the thermal resistance of static surface air films.

In a hot air oven it is not possible to make maximum use of these advantages because articles in the centre of a shelf are shadowed by surrounding items. Infra red ovens are designed to overcome this problem. One type consists of a horizontal wire-mesh conveyor belt that travels through an insulated tunnel containing infra-red heating elements (Fig. 29.8). Shallow trays each carrying a single layer of syringes are loaded on to the belt and receive a sterilising exposure of 20 minutes at 180°C as they pass through the tunnel. This exposure is well in excess of the recommendation of 7.5 minutes at 180°C made by Darmady, Hughes and Jones (p. 310) and, sometimes, rather shorter times (e.g. 10 min) are used. The arrangement of the elements and the absence of shadowing (because of the single layer) ensure that the heat is received evenly by the load, therefore, heating-up is very fast (cf. the hot-air oven) and the overall processing time is considerably reduced.

The amount of heat absorbed by and, therefore, the temperature produced in an irradiated object depends on—

- The distance from the source. The load should be close to the elements, as in the conveyor oven.
- The nature of the irradiated material. This influences the relative amounts of radiation absorbed, reflected and transmitted—
  - Surface colour. Black surfaces absorb well and, therefore, heat up much more quickly than bright shining surfaces that reflect strongly.

- (b) Surface character Smooth surfaces reflect more than rough surfaces
- (c) Type of material Glass reflects only a little radiation but it transmits a great deal, metals reflect in accordance with their surface colour and character but transmission is virtually nil because all the non reflected energy is absorbed in the surface layer

In a conveyor oven the heat is produced in the exposed upper surface of each article and then transferred by conduction and convection to the rest of its mass. During the exposure period equal areas of surface receive the same amounts of energy. However, a surface intensity sufficient to give a sterilising exposure to a particular syringe will under or over expose syringes in which the surface to mass ratio is smaller or larger respectively. For a similar reason, a syringe may receive a sterilising treatment if exposed in a container with a surface that absorbs well, but not if the surface is a powerful reflector. These difficulties indicate a major disadvantage of the conveyor oven, it is designed to give the correct exposure to a particular load and any variation in the latter may result in under or over exposure of some of the items. Within narrow limits this can be overcome by using containers with different types of surfaces, e.g. small, medium and large syringes can be packed in bright, dull and black aluminium tubes respectively when the smallest will absorb the least and the largest the most heat. A technique of this kind has been successfully used by Darmady *et al.* (1961).

Glass or aluminium containers are permissible. With aluminium the absorbed heat is very rapidly conducted through the tube and the surface is easy to treat if required. Glass transmits some of the radiation to its contents but is a poor conductor and therefore, there may be a large temperature difference between the upper and lower surfaces of the syringe, which causes strain and sometimes breakage in glass-metal types.

Other advantages of infra red sterilisation, for syringes, are—

- 1 The process is continuous. In the oven described above 600 small or 360 medium-sized syringes can be treated in an hour. Therefore, the method is very suitable for a syringe service.
- 2 Once exposure has begun articles cannot be removed before they have received adequate treatment (cf. the hot air oven).
- 3 The oven is simple in design and easy to use and maintain.

An interesting disadvantage is the difficulty of

determining internal temperatures accurately because the absorption, reflection and transmission characteristics of sensing devices generally differ widely from those of the article being heated.

In hospitals, the routine method of checking the process is by type IV Browne's tubes (see chapter 22) which are inserted into the barrel or a hollow plunger of a dummy syringe.

#### (c) Gas Ovens

An alternative apparatus for continuous syringe sterilisation is the forced convection gas oven (Harris, 1961; Patrick, Wharton, Prentis and Signy, 1961). It is a horizontal type of fan-circulated hot air oven incorporating a conveyor system and heated by gas.

In the oven described by Harris the syringes are transported on a monorail conveyor and receive an exposure of 1 hr at  $180 \pm 2^\circ\text{C}$ . The long time compared with the time normally used in an infra red oven is to ensure destruction of all micro-organisms, including highly resistant non-pathogenic spore-formers. The infra-red exposure is based on the thermal resistance of the spores of the most resistant pathogens. The different viewpoints represented by these two practices have been discussed in chapter 22 (see also Darmady, Hughes and Jones, 1958).

Advantages of the convection oven over the infra-red oven include—

- 1 Greater control of the sterilisation temperature.
- 2 No difficulty in sensing the temperature.
- 3 Lower cost, since gas is cheaper than electricity.
- 4 It can be used for mixed loads.

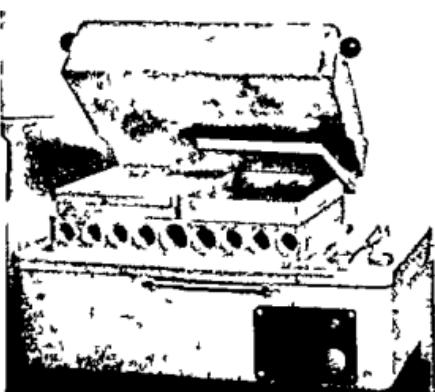


Fig. 299 CONDUCTED HEAT STERILISER  
(Courtesy Tunison and Mercer Ltd.)

**(d) Conducted heat Steriliser**

This method, suitable for small numbers of syringes, was developed by Darmady, Hughes, Jones and Tuke (1958)

The apparatus comprises a thermostatically controlled hot plate on to which is fixed an aluminium block with holes bored into one side to take six 2-ml, two 5 ml and one 10-ml syringe containers. A flat, lidded tray, for instruments, rests on the block and both are insulated by a fibre-glass cover (Fig 299)

Heat from the block is conducted through the syringe containers and the tray to their contents. The temperature variation is remarkably low, only  $\pm 1^{\circ}\text{C}$ , and at  $190^{\circ}\text{C}$  the syringes are sterilised in 22 minutes

**Syringes for Special Purposes**

Syringes used for certain materials must be kept separate and given special treatment before the normal cleaning process. Examples are syringes that have contained—

- 1 Radioactive isotopes, these are decontaminated by appropriate techniques and afterwards checked with a radiation detector
- 2 Materials contaminated with pathogenic organisms, these are placed in lysol immediately after use
- 3 The tuberculins (diagnostic preparations used in immunology), these are kept separate at all stages of processing and issued only for the same injection and even the same strength of injection on each occasion. The active principle of these products is strongly adsorbed by glass and difficult to remove during cleaning, traces leached into (i) other injections, could have harmful results, and (ii) weaker strengths of the same preparation, could invalidate the diagnostic test

There is a strong case for disposable syringes for these and similar preparations

**METAL INSTRUMENTS****1. Steam under Pressure**

A major requirement in the sterilisation of instruments is the protection of cutting edges. Since oxidation is the chief cause of damage, treatment with steam under pressure is suitable if the air has been properly vented and the steam is dry saturated. In addition, the process must ensure that the load is dry on removal because considerable deterioration can result from the combined effects of air and moisture during a long period of post sterilisation drying.

High-vacuum sterilisers have the advantages of a short cycle and rapid cooling of the load during the post-vacuum but as there are no serious penetration problems with instruments the pre vacuum is not essential and a downward displacement steriliser is quite satisfactory. The MRC Report (1959) suggests the following procedure—

Admit steam to the jacket

Load and close the chamber

Admit steam to the chamber and allow it to vent vigorously until the internal temperature is approximately  $100^{\circ}\text{C}$

Close the vent and allow the rest of the air to bleed away

Expose for 15 minutes at  $121^{\circ}\text{C}$ . The highly conducting load heats up almost instantaneously

Shut off the steam supply and vent the chamber steam to the atmosphere

Open the door. The hot instruments dry immediately

**2 Infra red Sterilisation in High Vacuum**

Long heating and cooling in air are undesirable. The length of the process in the hot-air oven is a serious disadvantage. The infra red conveyor oven requires a constant load, and although this problem can be overcome to some extent by making up small packs that will receive the correct exposure, e.g. a few instruments in an aluminium container of appropriate size (Welch, 1961), the procedure is unsuitable for the large and variable instrument requirements of operating theatres, in addition, if temperatures much in excess of  $180^{\circ}\text{C}$  are reached damage may occur during cooling.

Recently, Darmady *et al* (1961) devised an infra-red vacuum oven. The instruments are loaded into a suitable chamber which is evacuated to 1 to 2 mm Hg abs and thus is maintained during heating, which is by infra-red heaters on the roof and floor. One of the advantages of infra red over other methods of heat transfer is that no carrier is required and, therefore, it can be used in a vacuum. A short exposure (7 minutes) at a very high temperature ( $280^{\circ}\text{C}$ ) is given, and sterile nitrogen is admitted during cooling. The use of vacuum gives quicker heating up (no heat is lost to the air) and greater temperature stability (there are no convection currents), it also minimises oxidation, as does the nitrogen.

**CLEANING**

Complete removal of strongly adherent debris is assisted if instruments can be disassembled or opened fully (cf different types of scissors in the latter respect). Freedom from oil or grease is necessary if

steam sterilisation is used Ultrasonic cleaning is successful (see 'Syringes')

### RUBBER GLOVES

These are particularly difficult to sterilise satisfactorily because rubber is—

**1 Impervious to Steam** The method of packing must encourage steam penetration to the insides of the fingers and keep the surfaces apart as far as possible

The wrists are turned back, so that the outside need not be handled when the gloves are put on, and gauze pads are inserted in the positions shown in Fig 29 10a. Each matched pair is placed in a fabric envelope held loosely together with tapes (Fig 29 10b, c), and to minimise contact between the surfaces the envelopes are sterilised on their edges, in a single layer, in a flat perforated tray or, preferably, in a toast rack like holder inside a modern type of dressing box (p 339)

**2 Easily oxidised with loss of elasticity** Dry-heat sterilisation is highly destructive. For many years it was considered necessary to restrict the use of steam under pressure to the unsafe exposure of 15 minutes at 109°C (5 lb/in.<sup>2</sup>g), (Trillwood, 1957). However, the main factor responsible for the oxidative damage during safe exposures was later found to be, not the sterilisation time or temperature, but the long drying period made necessary by badly designed and/or operated sterilisers (M.R.C. Report, 1959). In modern equipment gloves can be exposed to 121°C for 15 min or, in a high vacuum steriliser, 134°C for 3 min without a significant shortening of their normal life

Ethylene oxide sterilisation is popular in America, and successful experiments have been carried out with ionising radiations particularly when the plastic

envelopes used as containers were vacuum sealed to minimise the effects of oxygen (Oliver and Tomlinson, 1960)

### CLEANING AND INSPECTION

Many detergents adversely affect rubber, and warm liquid soap is usually recommended. After careful rinsing the gloves are dried in clean air at room temperature. Then each glove is tested for perforations, often by inflating each finger in turn and holding it near to the face to detect escape of air

### GLOVE POWDERS

To assist insertion of the hands gloves are lubricated with a dusting powder before use

Talc, because of its excellence as a lubricant, was used exclusively for many years but slowly-accumulated evidence has shown that if it escapes into the tissues, through perforations in the gloves, it can cause irritation to which the body may respond by an overgrowth of cells, i.e. a non-malignant tumour (a granuloma). Serious results, including death from intestinal obstruction, have been attributed to these growths. Their cause was not discovered for some time because talc particles are not easily detected histologically unless the sections are examined under polarised light (Graham and Jenkins, 1952 a, b).

Now, talc is being replaced by absorbable glove powders. An ideal glove lubricant should be—

- (a) Non toxic and non-irritant.
- (b) Completely absorbed by the tissues
- (c) Unaffected by steam sterilisation
- (d) Harmless to rubber
- (e) A good lubricant

A number of preparations has been developed (see Elliott, 1953; Whittet and Fairburn, 1949, 1952) based on alginates, mineral compounds or treated gelatin, but the most popular types contain modified starch, e.g. Absorbable Glove Powder B P

When starch is treated with certain reagents, e.g. formaldehyde or epichlorohydrin, it no longer gels on heating and can be dry or steam sterilised. To improve the flow properties traces of light magnesium oxide are added (Craik, 1958; Craik and Miller, 1958). The powder is usually sterilised in bulk, by hot air or ethylene oxide, and then distributed into small envelopes that are autoclaved with the gloves

### CATGUT

Heat and chemical methods for the sterilisation of catgut are described by Cooper and Gunn (1957a). Sterilisation by ionising radiation has already become established as a satisfactory alternative (Owen

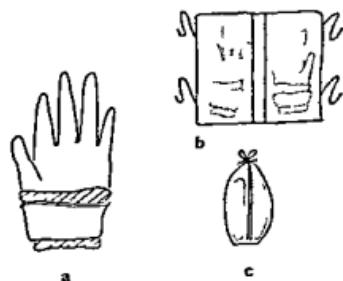


Fig. 29 10 PACKAGING OF RUBBER GLOVES

Dawson, 1962) Among its advantages are—

- 1 Smaller loss of tensile strength
- 2 The container is safer to handle. The glass tubes used for heat and chemically sterilised gut may cut the gloves and fingers of the theatre staff and produce spicules that can damage the thread and even find their way into the operation field. These risks are absent with the sachets of plastic-aluminium foil laminate used for radiation sterilisation.
- 3 The gut is easier to use because it is only loosely coiled in the sachets.

### ABSORBABLE HAEMOSTATS

These are spongy or fibrous materials used to stop bleeding. They are of particular value in surgical operations that carry a serious post operative risk of internal haemorrhage, e.g. the suturing of blood vessels. They are packed round the lesion, where they provide a large rough surface that encourages platelet fracture, an essential preliminary to blood clotting. They are absorbed in about six weeks. The B.P. includes three examples.

#### 1. Fibrin Foam

Fibrinogen, obtained from human blood, is dissolved in Sodium Chloride Injection and whipped into a foam which is then clotted (i.e. converted into fibrin) by the addition of human thrombin. The foam is freeze-dried (Cooper and Gunn, 1957b) and, since heating in the presence of moisture denatures the fibrin, is sterilised by dry heat at 130°C for 3 hr.

#### 2 Absorbable Gelatin Sponge

A solution of pure gelatin containing a trace of formaldehyde is foamed, freeze-dried and sterilised by dry heat at 140°C. Moist heat completely destroys its physical properties.

#### 3 Oxidised Cellulose

This is prepared by the oxidation, with nitrogen dioxide, of cotton or regenerated cellulose in the form of gauze or lint. It retains the appearance of the original fabric. It cannot be heat sterilised and gaseous methods are used.

(For further information, see Betts and Whittet, 1962, Fairburn and Whittet, 1948, and Fish, 1959.)

### HYPODERMIC SOLUTION TABLETS

These are small tablets, made by moulding or light compression, that are dissolved in Water for Injection, usually in the syringe, immediately before use. Medicaments that have been marketed in this form include papaveretum, morphine salts and penicillin salts.

The preparation of moulded tablets (tablet triturations) is discussed in chapter 6. The hypodermic type must be completely and quickly soluble. Generally lactose is the diluent. Scighano, Purdum and Foss (1950) recommend the addition of 0.1 per cent of sodium metabisulphite to tablets of morphine salts to prevent discolouration, and the use of 3 per cent alcohol and 5 per cent sucrose in distilled water as a moulding agent. Moulding and compression (see chapter 8 and Stephenson, 1950) must be carried out under aseptic conditions.

Walters, Christenson and Kreider (1953) invented an apparatus for determining the solubility of moulded solution tablets. A test of this kind must show that the tablet dissolves rapidly at room temperature in a small volume of water without vigorous shaking.

Cherryman and Vickers (1957), after studying the packaging solubility and sterility of a number of commercial types, concluded that, whenever possible, they should be replaced by solutions in ampoules or multi-dose containers.

Packaging requires particular care. A common method is to include several tablets in a narrow, glass vial plugged with cotton wool and sealed with a waxed cork or, more recently, a plastic plug. Removal of a single tablet is difficult without contaminating the rest and, if the cotton wool is replaced, fibres can be trapped between tube and closure forming a tiny wick that carries moisture inside.

### LAMELLAE

Lamellae (eye discs) are used occasionally when very accurate medication of the eye is desired. They are about  $\frac{1}{8}$  in. in diameter and contain the medicament in a glyco-gelatin base. A clean, moist camel hair brush is used to place one on the inner surface of the lower eyelid where it quickly dissolves.

Using aseptic technique throughout, a glyco-gelatin base is prepared and strained through well-washed muslin. The medicament, dissolved in the smallest possible volume of water, is added and mixed. The mixture is poured on to a warm waxed sheet of plate glass and spread evenly over an area defined by a square paper pattern. The film is dried at not more than 36°C and then separated and cut into discs, e.g. with a sharp cork borer.

The British Pharmaceutical Codex includes Lamellae of Atropine, and Cocaine with Homatropine.

### IMPLANTS

These small tablets, used for depot therapy, are made by heavy compression or fusion (see p. 474).

The compressed tablets are prepared by tabletting

sterile crystals in a sterile machine with full aseptic precautions

The fused type are made by holding the substance at 5° to 10°C above its melting point until it is completely molten. Then it is filled into moulds to produce individual tablets. Alternatively, a long cylinder may be cast and later divided into implants of the correct weight (Edkins and Tindall, 1953). This method renders the interior sterile, the outside is sterilised by immersion in 0.002 per cent phenyl-mercuric nitrate for 12 hr at 75°C (boiling causes softening), afterwards the surface is dried with sterile filter paper.

Some steroids are very stable to heat (e.g. the medicaments of the official oily depot injections) and, therefore, in a few cases, where the treatment does not affect the structure of the implant, dry heat sterilisation in the final container is possible.

Implants must be packed singly. Glass tubes are used, and sometimes the tablet is wrapped in paper first. To prevent contamination from spicules during opening, and damage due to movement during handling and transport, a wool plug is placed on each side of the pellet, the tube should be opened across one of these plugs.

#### JELLIES AND CREAMS IN COLLAPSIBLE TUBES

If this type of preparation is autoclaved the tube bursts and part of the contents is lost. On a small scale the problem is solved by autoclaving the product in a screw-capped bottle and then, after thorough shaking, pouring it under aseptic conditions, just before it loses its fluidity, into a dry heat sterilised tube which is then sealed aseptically.

Lachman, Jaconia and Eisman (1959) modified an autoclave to permit the sterilisation of jellies in tubes. The adaptation allowed the introduction of air under pressure to create a total pressure (air + steam), during sterilisation and cooling, in excess of the vapour pressure within the containers. An adequate partial pressure of steam was maintained to provide a sterilising temperature.

#### CULTURE MEDIA

Important aims in the sterilisation of culture media are the protection of essential nutrients, the prevention of caramelisation of sugars and the preservation of a high degree of clarity.

These are best fulfilled by filtration through a bacteria proof filter but the complexity of this method and its unsuitability for viscous preparations tend to restrict its use to particularly heat sensitive media and concentrated sugar solutions.

Whenever possible autoclaving is preferred and when modern equipment, in which heating up and cooling down are reduced to a minimum, is used, an exposure of 121°C for 10 to 15 minutes is practicable in many cases. The sterilisation of media in bulk (in screw-capped bottles) is a potential application for the quick-cooling sterilisers used for bottled fluids. With other autoclaves small containers that will cool rapidly are essential.

Screw-capped bottles are preferable to tubes because there is no risk of concentration from evaporation. Air pocketing between containers is prevented by packing in baskets or perforated trays. Repeated sterilisation should be avoided, e.g. sterilisation in bulk followed, at a later date, by distribution into smaller containers and re-sterilisation.

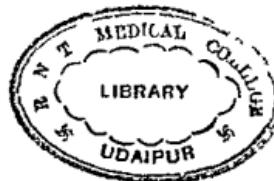
Occasionally, the high temperatures of steam sterilisation are harmful and Tyndallisation is used. The main criticism of this method (see chapter 22) does not apply when it is used for culture media because a nutritive environment is available for spore germination between the heatings.

Thermolabile media have been successfully sterilised in about 6 hr by the addition of 0.5 to 1 per cent of chilled liquid ethylene oxide. There was no evidence of interference with essential metabolites, and a day later the broths were fully capable of supporting bacterial growth, the bactericide had been destroyed by combination with water (to form ethylene glycol) and organic acids, (Wilson and Bruno, 1950).

#### REFERENCES

- 1 BETTS, T. J. and WHITIET, T. D. (1962) A new absorbable haemostatic—its uses and identification. *Pharm J.*, 188, 269-270.
- 2 BRADLEY, T. J., MORTON, I. K. and FRAME, E. A. (1959) Ultrasonics in the syringe service. *Publ Pharm.*, 16, 110-116.
- 3 BRITISH STANDARD 1263 1946 *Hypodermic syringes for use in medical and surgical practice*. British Standards Institution, London.
- 4 BRITISH STANDARD 1619 1962 *Hypodermic syringes for insulin injection*. British Standards Institution, London.
- 5 CHERRYMAN, E. W. and VICKERS, J. A. (1957) Hypodermic solution tablets. *Pharm J.*, 178, 299.
- 6 COOK, I. J. Y. (1958) A laboratory operated syringe service. *J. med. Lab. Tech.*, 15, 280-282.
- 7 COOPER, J. W. and GUINN, C. (1957a) *Tutorial Pharmacy* 5th, Ed. Pitman, London, 672-673.
- 8 COOPER, J. W. and GUINN, C. (1957b) *ibid.*, 79-83.
- 9 CRAIK, D. J. (1958) The flow properties of starch powders and mixtures. *J. Pharm. Pharmacol.*, 10, 73-79.

- 10 CRAIK, D J and MILLER, B F (1958) The flow properties of powders under humid conditions *J Pharm Pharmacol.*, 10, 1367-1447
- 11 DARMADY, E M, HUGHES, K E A and JONES, J D (1958) Thermal death times of spores in dry heat *Lancet*, 2, 766-769
- 12 DARMADY, E M, HUGHES, K E A, JONES, J D and TUKE, W (1958) Sterilisation by conducted heat *Lancet*, 2, 769-770
- 13 DARMADY, E M, HUGHES, K E A, JONES, J D, PRINCE, D and TUKE, W (1961) Sterilisation by dry heat *J clin Path.*, 14, 38-44
- 14 DAVIS, R W (1952) Economy in the central syringe service *Pharm J.*, 169, 403-404
- 15 EDKINS, R P and TINDALL, W J (1953) Implants of the steroid hormones *Alchemist, Leeds*, 17, 367-373
- 16 ELLIOTT, J R (1953) Lubricants for surgeons gloves *Alchemist, Leeds*, 17, 126-130
- 17 FAIRBURN, J W and WHITTET, T D (1948) Absorbable haemostatics, their uses and identification *Pharm J.*, 160, 149-150
- 18 FISH, F (1959) Newer surgical materials *Pharm J.*, 183, 49-53
- 19 FOWLER, P J (1953) Dangers in syringe lubricants *Pharm J.*, 170, 14
- 20 GRAHAM, J D P and JENKINS, M E (1952a) The effects of introducing certain suggested substitutes for talc into the peritoneal cavity and into wounds in experimental animals *J Pharm Pharmacol.*, 4, 392-398
- 21 GRAHAM, J D P and JENKINS, M E (1952b) Value of modified starch as a substitute for talc *Lancet*, 1, 590-591
- 22 GRAINGER, H S (1956) The sterile syringe service at Westminster Hospital, London *Publ Pharm.*, 13, 27-31
- 23 HADGRAFT, J W (1959) A central syringe service *M and B Pharmaceutical Bulletin*, 8, 66-69
- 24 HARRIS, M (1961) Industrial sterile supply services *Symposium on the sterilisation of surgical materials* The Pharmaceutical Press, London, 160-166
- 25 LACHMAN, L, JACONIA, D and EISMAN, P (1959) Protective effects of air under pressure on certain pharmaceuticals during autoclaving *J Amer pharm Ass., Sci Ed.*, 48, 541-547
- 26 MATTHEWS, D R (1957) Salvage of jammed syringes *Publ Pharm.*, 14, 94-95
- 27 MRC (MEDICAL RESEARCH COUNCIL) MEMORANDUM No 41 (1962) *The sterilisation, use and care of syringes* Her Majesty's Stationery Office, London
- 28 MRC (MEDICAL RESEARCH COUNCIL) REPORT (1959) *Report by the Medical Research Council Working Party on pressure steam sterilisers* Her Majesty's Stationery Office, London
- 29 MRC (MEDICAL RESEARCH COUNCIL) WAR MEMORANDUM No 15 (1945) *The sterilisation, use and care of syringes* Her Majesty's Stationery Office, London
- 30 MYERS, J A and GOODMAN, J E (1960) Cleaning of surgical instruments using a new type of ultrasonic apparatus *Pharm J.*, 185, 585-587
- 31 NUFFIELD REPORT (1957) *The planning and organisation of central syringe services* Nuffield Provincial Hospitals Trust, London
- 32 OLIVER, R and TOMLINSON, A H (1960) The sterilisation of surgical rubber gloves and plastic tubing by means of ionising radiation *J Hyg., Camb.*, 58, 465-472
- 33 OWEN DAWSON, J (1962) Surgical catgut *Pharm J.*, 188, 159-163
- 34 PATRICK, E A K, WHARTON, R H, PRENTIS, K and SIGNY, A G (1961) Sterilisation by gas ovens *J clin Path.*, 14, 62-65
- 35 RAINE, G (1958) Aluminium foil overseals *Publ Pharm.*, 15, 26
- 36 RICHARDS, J M and WHITTET, T D (1958) Nylon syringes under test *Chem and Drugg.*, 169, 16-18
- 37 ROBERTS, J G (1957) Development of a sterile syringe service *Pharm J.*, 178, 132
- 38 SCIGLIANO, J A, PURDUM, W A and FOSS, N E (1950) The manufacture, solubility and stability of hypodermic tablets containing morphine *J Amer pharm Ass., Sci Ed.*, 39, 627-629
- 39 STEPHENSON, D (1950) Hypodermic tablets *Pharm J.*, 164, 499
- 40 TRILLWOOD, W (1957) Rubber glove ritual *Pharm J.*, 178, 298
- 41 WALTERS, E L, CHRISTENSON, G L and KREIDER, H R Jr (1953) Solution times of hypodermic tablets *J Amer pharm Ass., Sci Ed.*, 42, 439-441
- 42 WELCH, J D (1961) The organisation of central supply departments *J clin Path.*, 14, 69-75
- 43 WHITE, J (1950) Sterile syringe container *Pharm J.*, 164, 440
- 44 WHITTET, T D and FAIRBURN, J W (1949) Absorbable glove powders, their uses and identification *Pharm J.*, 163, 421-422
- 45 WHITTET, T D and FAIRBURN, J W (1952) Absorbable glove powders, two new samples *Pharm J.*, 168, 343
- 46 WILSON, A T and BRUNO, P (1950) *J exp Med.*, 91, 449 Ethylene oxide for sterilisation (Abstracted in *J Pharm Pharmacol.*, 2, 731)



## Appendix 1

# Reducing Agents and Antioxidants

THE decomposition of pharmaceutical materials by oxidation may be kept in check by suitable methods of storage. These include storage in well-closed containers, storage under vacuum or inert gas, and storage at low temperatures. Such methods are not always practicable and therefore it is necessary to resort to the use of additives which will prevent or diminish oxidation. To understand the use of these additives it is necessary to understand how oxidation

occurs. It may be due to—

- (a) Atmospheric oxygen
- (b) Oxidising agents
- (c) Microbiological enzymes

Reducing agents are effective against (a) and (b)

Antioxidants are effective against (a)

Antiseptic preservatives are effective against (c)

## REDUCING AGENTS

These act by being preferentially oxidised and are gradually used up. Provided that a suitable excess of reducing agent is present an oxidisable active principle will be protected.

Examples in use in pharmacy include sodium sulphite, sodium metabisulphite, sulphurous acid and sulphur dioxide. In each of these cases the fundamental reaction is—



Other examples are hypophosphorous acid, dextrose and glucose.

Listed below are a number of examples of preparations in the *British Pharmacopoeia*, the *British Pharmaceutical Codex* and the *British National Formulary* in which reducing agents are used. The percentages indicate the concentration of reducing agent.

### PREPARATIONS IN WHICH SODIUM METABISULPHITE IS USED

Adrenaline Injection B P 0.1% w/v

Adrenaline Solution B P 0.1% w/v

Lignocaine and Adrenaline Injection B P 0.1% w/v

Procaine and Adrenaline Injection B P 0.1% w/v

Procainamide Injection B P 0.1% w/v

Morphine Sulphate Injection B P 0.1% w/v

Injection of Morphine and Atropine B P C 0.1% w/v

Injection of Noradrenaline B P C 0.1% w/v

Spray of Adrenaline and Atropine, Compound B P C 0.114% w/v

Spray of Isoprenaline B P C 0.1% w/v

Spray of Isoprenaline, Compound 0.1% w/v

Eye Drops of Physostigmine B P C 0.04% w/v

Mixture of Sodium Salicylate B P C 0.114% w/v

Syrup of Raspberry B P C = 350 p p m w/w of sulphur dioxide

Sodium Salicylate Mixture, Strong B N F 0.114% w/v

### PREPARATIONS IN WHICH SULPHURIC ACID IS USED

Syrup of Blackcurrant B P C 350 p p m w/w of sulphur dioxide

Syrup of Raspberry B P C 350 p p m w/w of sulphur dioxide

### PREPARATIONS IN WHICH HYPOPHOSPHOROUS ACID IS USED

Mixture of Ferrous Sulphate B P C 2.08% v/v of B P C dilute acid

Mixture of Ferrous Sulphate for Infants B P C 2.5% v/v of B P C dilute acid

Solution of Ferrous Iodide B P C. 8% v/v B P.C.  
dilute acid

Syrup of Ferrous Iodide 10% v/v of B P C. dilute  
acid

(Dilute Hypophosphorous Acid B P C. contains 10%  
w/w of Hypophosphorous Acid )

#### PREPARATIONS IN WHICH DEXTROSE IS USED

Carbachol Injection B P. 50% w/v

Injection of Bismuth B P C. 50% w/v

#### PREPARATION IN WHICH LIQUID GLUCOSE IS USED

Pills of Ferrous Carbonate B P C

## ANTIOXIDANTS

Because these are effective against oxidation by atmospheric oxygen (autoxidation), it has been suggested that a more suitable name would be anti-oxidents' and this name is frequently quoted in the literature on the subject

Unlike reducing agents, they are not preferentially oxidised but act by blocking an oxidative chain reaction. Consequently they are effective in low concentration and are not used up to any great extent in the process

Their commonest role is in the preservation of fixed oils and fats which are particularly susceptible to oxidation, with the formation of objectionable degradation products of unpleasant odour and taste. This oxidised condition is called rancidity and it develops in a characteristic fashion in two stages

- (a) An induction, or lag period in which decomposition is slow the rate varying with the presence of natural inhibitors of oxidation or, on the other hand, the presence of pro-oxidant substances
- (b) A stage when the reaction rate increases logarithmically

Rancidity may be due to either—

- 1 Oxidation by atmospheric oxygen
- 2 Oxidation by enzymes

#### 1 RANCIDITY DUE TO ATMOSPHERIC OXIDATION (AUTOXIDATION)

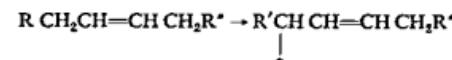
Rancidity may readily be noticed by the sensory characters of taste and smell, but the first products of rancidity detected by chemical tests are hydroperoxides. Further degradation of these hydroperoxides produces aldehydes and esters which are characteristic of rancidity. Lea (1953), in a review of anti-oxidants, gives an account of these degradation products in schematic form.

Many theories have been advanced to explain the autoxidation of oils and fats and the role of anti-oxidants in its prevention. It is clear, however, that anti-oxidants act at a very early stage of the oxidation, since they prevent the formation of peroxides which

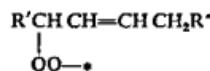
are the first detectable products. Also, since quite low concentrations are effective they do not act like reducing agents which become oxidised and are used up comparatively quickly

The most acceptable theory is explained on the basis of a chain reaction due to a free radical mechanism. This may be explained as follows—

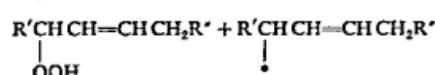
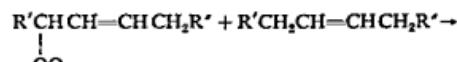
A free radical is formed by the loss of a hydrogen atom commonly from the methylene group adjacent to a double bond. This may be initiated by agencies such as heat, light, trace metals such as copper, nickel, manganese or iron, or other pro oxidant impurities



Atmospheric oxygen converts this free radical into a peroxide which is also a free radical—



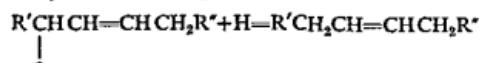
The peroxide radical now reacts with another molecule of the original oxidisable substance to produce (a) a hydroperoxide and (b) another free radical—



The free radical, thus produced, takes up oxygen as before and the chain reaction proceeds

The hydroperoxides decompose to produce the undesirable aldehydes and esters

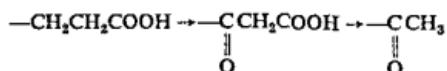
Antioxidants act by stopping the chain reaction at its first stage by supplying a hydrogen atom, thus destroying the free radicle and preventing it from being converted to a peroxide—



In support of this theory it is well known that the ortho and para derivatives of dihydric and polyhydric phenols are among the most effective antioxidants and they are capable of donating a hydrogen atom by undergoing keto-enol tautomerism

## 2 RANCIDITY DUE TO ENZYME ACTION

Enzymes present in the oxidisable material or in micro-organisms which have gained access to it may cause rancidity. Some produce oxidative effects similar to atmospheric oxidation but others attack saturated fatty acids at the  $\beta$  carbon atom producing ketonic acids and methyl ketones—



This is referred to as 'ketonic rancidity' in distinction from oxidation due to atmospheric oxygen which is called oxidative rancidity'

Where micro-organisms are involved, sterilisation with aseptic processing and storage may be employed. As this is not always practicable antiseptic preservatives may be used, but it should be remembered that fatty foods such as butter and margarine, which are consumed regularly, might involve ingestion of undue quantities

## THE REQUIREMENTS OF AN IDEAL ANTIOXIDANT

- 1 Effective in providing a satisfactory degree of protection in low concentration
- 2 Non toxic and non irritant and form no harmful products
- 3 Readily soluble or dispersible in the medium
- 4 Possess no objectionable colour, odour or taste
- 5 Compatible with other constituents

## CLASSIFICATION OF ANTIOXIDANTS

Various classifications have been suggested, including those of Olcott and Mattill (1936), Olcott (1941) and Bergel (1944). The student should also refer to the review articles by Shotton (1954) and Weir (1955). Bergel's classification divides antioxidants into four classes, viz 1 Quinol group 2 Pyrogallol group 3 Dienols, and 4 Amines. Substances called syn-

ergists are sometimes classed as antioxidants. In the following lists they are grouped separately

### Synergists

These are substances which increase the effectiveness of antioxidants. Some like ascorbic acid are claimed to have antioxidant properties of their own but generally they act by enhancing the effect of the antioxidant. Their method of action is not always clear but in some cases this is, no doubt, due to the sequestering of metallic ions which are active pro-oxidants. Many synergists are water soluble acids such as citric acid, but others such as mono-isopropyl citrate are oil soluble. The use of synergists would appear to be of particular value in the case of emulsified fats and oils. Here the large surface area exposed and the presence of entrained air renders the oil more susceptible to oxidation. The reaction of the aqueous phase is important. In alkaline solution, for example, the simpler polyphenols are valueless as antioxidants since they are unstable and too water soluble. In this connexion the water soluble acid synergists are valuable. Oil soluble synergists are also effective in acid but not in alkaline emulsions. A valuable review on these matters and other aspects of antioxidants is given in The First Documentary Edition of the *American Perfumer*

### SOME COMMON ANTIOXIDANTS AND SYNERGISTS

#### *Quinol Group*

Hydroquinone

Tocopherols

Hydroxychromans

Hydroxycoumerans

Butylated Hydroxy Anisole (B H A)

Butylated Hydroxy Toluene (B H T)

#### *Catechol Group*

Catechol

Pyrogallol

Nordihydroguaiaretic Acid (N D G A)

Gallic Acid

Ethyl Gallate

Propyl Gallate

Octyl Gallate

Dodecyl Gallate

#### *Nitrogen containing Substances*

Alkanolamine esters ( $\text{ROOC CH}_2\text{NR}'\text{R}''$ )

Amino and hydroxy derivatives of *p* phenylamine-diamine

Diphenylamines

Caseine and Edestine

*Sulphur-containing Substance*

Cysteine Hydrochloride

**SYNERGISTS***Water Soluble*

Citric Acid

Tartaric Acid

Phosphoric Acid

Carboxymethylmercapto-succinic Acid

Ascorbic Acid

*Oil Soluble*

Ascorbyl Palmitate

Mono-isopropyl Citrate

Palmityl Phosphate

Mono-stearyl Citrate

The following is a brief account in each case of some of the better known antioxidants

**Quinol Group**

*Hydroquinone* Hydroquinone is soluble in water but only slightly soluble in fats and oils. It has been used to preserve halibut and shark liver oil, successfully protecting the Vitamin A content. It has been claimed to be better than benzoin for the preservation of lard in as low a concentration as 0.005 per cent.

It is used to stabilise ether (0.1 per cent), paraldehyde (0.05 per cent) and peppermint oil (0.1 per cent).

*Tocopherols* The tocopherols  $\alpha$ ,  $\beta$  and  $\gamma$  occur in wheat and maize germ oils and appear to act as natural antioxidants. Their effect seems to be enhanced by the presence of other substances as their antioxidant properties are greatly reduced when extracted.

Liquid Paraffin, although reasonably inert, is subject on long storage to autoxidation with the production of, first, peroxides, and then of aldehydes and organic acids, with the development of an unpleasant odour and taste. This may be prevented by the use of tocopherol (Salzman and Sonneborn, 1951) and the *Pharmacopœia* permits the use of 10 parts per million.

Related compounds, such as the hydroxchromans, have been found of value in the preservation of Vitamin A in oily solution or in an emulsion of halibut liver oil.

*Butylated hydroxy anisole (B H A)* This compound is widely used in Britain, Canada and the U.S.A., and is officially recommended by the Minis-

try of Food (1958) for the protection of fats, fixed and essential oils. Fat solubility is increased by the tertiary butyl side chain in the *ortho* or *meta* positions to the hydroxy group and this also ensures a satisfactory 'carry over' effect into cooked preparations. Acid synergists are commonly used with it. The Ministry of Food recommends the use of 0.02 per cent for fats and fixed oils and 0.1 per cent for essential oils.

Clark and Kitchen (1960) have shown that Wool Alcohols may effectively be protected against oxidation by 500 p.p.m. of B H A.

*Butylated hydroxy toluene (B H T)* This is similar to butylated hydroxy anisole in action. It has the advantage that at raised temperatures it is odourless while butylated hydroxy anisole has a somewhat objectionable odour.

**Catechol Group**

*Catechol* is very water soluble and is limited in use because it gives a smoky flavour to fats and fixed oils. It has been used for the quantitative assessment of antioxidants, the 'Catechol Index' being the ratio of activity of an antioxidant to that of an equimolar concentration of catechol.

*Nordihydroguaiaretic acid* This substance has been shown to be an effective antioxidant for fats and oils. It has been used for the preservation of lard (0.005%) but it imparts a detectable flavour to butter. Synergists increase its effect, particularly lecithin and, to a lesser extent, citric and tartaric acids.

*Pyrogallol* is sometimes used to stabilise ether but its use for fats and fixed oils is limited because it causes considerable darkening in colour and is not free from toxicity.

*Gallic acid* is also inclined to discolour preparations although it has been used to stabilise paraldehyde. Its low oil solubility limits its value as an antioxidant for fats and fixed oils but its esters, which are more oil soluble, are among the most important antioxidants available.

*Esters of Gallic acid* Ethyl, propyl, octyl and dodecyl gallates are all used as antioxidants. Fat solubility increases with chain length, ethyl gallate being more water than oil soluble and least valuable of those mentioned. It also has a somewhat bitter taste, no doubt due to its water solubility. The higher members are comparatively tasteless. Propyl, octyl and dodecyl gallates are antioxidants permitted by the Ministry of Food. The recommended concentrations are 0.01 per cent for fats and fixed oils and 0.1 per cent for essential oils. Lard of the *British Pharmaceutical Codex* contains a suitable

antioxidant 'such as 0.01 per cent of propyl gallate' Propyl gallate is extensively used in Britain, Europe, Canada and the U.S.A. It is one of the most popular antioxidants because of its undoubted effectiveness and lack of toxicity or unpleasant sensory characters Recently, considerable interest has been shown in octyl and dodecyl gallates, and it would seem that they are at least as effective and acceptable as the propyl ester

#### Nitrogen-containing Substances

These are of less importance in the food and pharmaceutical industries, but a number of amines have been used with success in the preservation of rubber

Diphenylamine has been used to preserve ether and paraaldehyde, and the amino and hydroxy derivatives of *p*-phenylenediamines have been used to stabilise Vitamin A preparations

Alkanolamine esters of the general formula  $\text{ROOC CH}_2\text{NR}'\text{R}''$ , are effective antioxidants for fats and fixed oils

#### Sulphur-containing Compounds

*Cysteine hydrochloride* is to some extent capable of preventing the oxidation of Vitamin C in aqueous solution Glutathione is more effective for this purpose being capable of chelating copper which is an effective pro oxidant

#### Naturally Occurring Substances

These include such well known substances as the tocopherols, Siam benzoin and guaiacum resin The tocopherols have already been mentioned Guaiacum has been used in America as a preservative for lard Siam benzoin is of particular interest to us as it has been used as a lard preservative for pharmaceutical use Benzoinated Lard was formerly a pharmacopoeial preparation made by digesting Siam benzoin in lard at a raised temperature and filtering the product The constituent responsible for preserving the lard is said to be coniferyl benzoate (Brindle and Pedley, 1942)

#### Evaluation of Antioxidants

Rancidity in oils is expressed by a 'peroxide number' 100 mEq. of peroxide per 1,000 G of sample is considered the maximum amount of peroxide that can be present in a fat or oil without developing a rancid odour and taste Various chemical methods of determining the peroxide content are used and need not be described here A sample containing an antioxidant and a control are heated at 210°F while a stream of dried purified air is blown through it The ratio of the time in hours required to produce rancidity in the two samples indicates the efficiency of the antioxidant

#### REFERENCES

- BERGEL, F (1944) Some Aspects of the Chemistry of Antioxidants *Chem and Ind*, 14, 127-128
- BRINDLE, H and PEDLEY, E (1942) The Preservation of Lard for Pharmaceutical Use *Quart J Pharm Pharmacol*, 15, 389
- CLARK, E W and KITCHEN, G F (1960) A Note on Autoxidation and its Inhibition in Wool Alcohols *B P J Pharm Pharmacol*, 12, 233-236
- FOOD STANDARDS COMMITTEE REPORT (1954 reprinted 1960) *H M Stationery Office*
- LEA, C H (1953) Recent Developments in the Study of Oxidative deterioration of Lipids *Chem and Ind* 1303 1309
- OLCOTT, H S (1941) Antioxidants for Edible Fats and Oils *Oil and Soap*, 18, 77-80
- OLCOTT, H S and MATTILL, H A (1936) Antioxidants and the Autoxidation of Fats VII Preliminary Classification of Inhibitors *J Amer chem Soc*, 58, 2204-2208
- SHOTTON, E (1954) Antioxidants *Pharm J*, 173, 297-298
- STAT INST (1958) No 1454 The Antioxidants in Food Regulations
- WEIR, R F (1955) Antioxidants in Foods and Drugs *Pharm J*, 174, 311-312
- ZALZMAN, L and SONNEBORN, H (1951) Stabilisation of Medicinal White Oil *Bull Natl Form Comm*, 19, 41-45



## Appendix 2

### Pyrogen Tests

#### THE BRITISH PHARMACOPÆIA TEST FOR PYROGENS

This test is based on the rise in body temperature of rabbits when the preparation is injected intravenously.

The temperature is measured in the rectum with a clinical thermometer, a thermocouple or a probe containing a thermistor bead.

##### 1 Thermometers

This is the least complicated method but the repeated disturbance of the rabbits may affect their temperature. Nursing on the operator's lap has been found to prevent this.

##### 2 Thermocouples

This method is more convenient and less time-consuming. It allows continuous recording without disturbing the rabbits because the thermocouples can be left *in situ* throughout the test. The apparatus consists of a thermocouple for each animal and a common reference junction in a very accurately controlled thermostatic water bath. Each thermocouple can be switched into circuit as required, and the current, hence the temperature, measured on a galvanometer (Fig. A2 1).

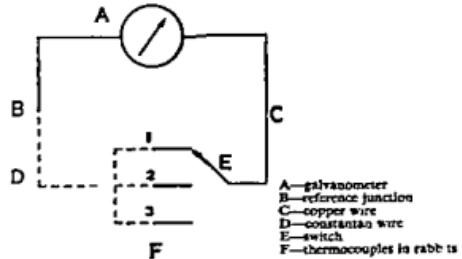


Fig. A2 1

##### 3. Thermistor Beads

These are tiny beads of a material that decreases rapidly in resistance with rising temperature. They have several advantages over thermocouples of which one is that no reference temperature is required. They are set up in an arrangement like a Wheatstone bridge (Fig. A2 2). The change in thermistor resistance caused by the temperature change of the rabbit is balanced by altering the variable resistance. Often, in this and the previous method, apparatus is used that, at suitable time intervals, automatically records the temperatures on a chart.

Healthy rabbits of either sex and not less than 1.5 kg in weight are used. The white Himalayan strain is often preferred because of its high sensitivity to pyrogen, but it is not amenable to nursing. No rabbit is used if

(a) It has an abnormally high body temperature (i.e. greater than 39.8°C) or

R<sub>1</sub>—thermistor in rabbit  
R<sub>2</sub>—variable resistance  
R<sub>3,4</sub>—fixed resistors

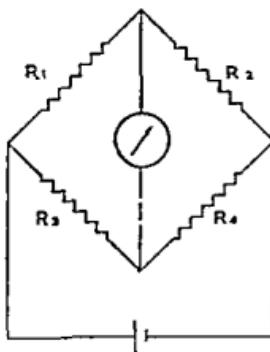


Fig. A2 2

(b) It has taken part in a positive test during the previous three weeks or a negative test in the previous three days, this is to prevent the reduction in response that occurs when pyrogen is given frequently. Also, if a rabbit has not been used previously, or for the past two weeks, a 'sham' test, using pyrogen free isotonic saline, is performed one to three days before the test proper, animals showing an abnormal rise or fall in body temperature are excluded from pyrogen tests until they behave normally. This pre testing also introduces them to the test conditions.

A rabbit's temperature may be raised by noise, disturbance, a high room temperature and food intake. Consequently, the test is carried out in a quiet room having a temperature within 3°C of the rabbits' living quarters, or in which they have been acclimatised for at least 18 hr. Food is withheld overnight and until the test is over and no water is given during the test.

The thermometer or other temperature sensing device must be inserted to a standardised distance because, within limits, the deeper the insertion the higher the temperature. The *Pharmacopœia* suggests 6 to 9 cm but the important thing is to keep the distance constant for any one rabbit in any one test. Because of this, if an 'electrical thermometer' is used it must be tied to the rabbit's tail and the animal restrained to prevent it from dislodging the instrument. The form of restraint should allow the rabbit to sit comfortably, and it usually consists of a box with a loose-fitting head-stock.

The test is first carried out on 3 animals, which are put in their boxes for  $\frac{1}{2}$  to 1 hr before the test begins. Temperatures are recorded at regular intervals of not more than 30 min beginning at least 90 min before injection and continuing for 3 hr afterwards. The injection is given intravenously, usually into an ear vein, if its volume is more than 10 ml it is warmed to 30 to 40°C previously. Pyrogens must not be introduced from glass vessels or syringes and, therefore, these must be depyrogenised, 250°C (dry heat) for 40 min has been found suitable.

The response of each rabbit is found by subtracting the mean of the temperatures recorded in the 40 min immediately preceding injection from the highest temperature afterwards. Then the responses of the 3 rabbits are added to give the 'summed response'. The result is obtained from a table in the *British Pharmacopœia*, the material passes if the summed response does not exceed a specified value and fails if it is greater than a second, higher, value. If the

summed response falls between the two values the test is repeated and the six results summed, this is continued, if necessary, up to a total of four groups of rabbits (i.e. 12 results in all).

### PYROGEN TESTING BY LEUCOCYTE COUNTS

The injection of pyrogen causes changes in the white cell picture (e.g. a fall in small lymphocytes and a rise in young neutrophils). These have been used as indicators of pyrogenic activity and further details will be found in the papers listed below.

### QUANTITATIVE ESTIMATION OF PYROGENICITY

The *British Pharmacopœia* test for pyrogens is a limit test—it merely ensures that the amount of pyrogen in solutions for injection is insufficient to cause a pyrogenic reaction.

In recent years attempts have been made to estimate pyrogens quantitatively using methods based on the biological effects used for testing, i.e. temperature rise and leucocyte changes, particularly the former.

An essential for a satisfactory biological assay is a stable preparation for use as a standard, and much work is in progress on the preparation, stability and activity of possible standard pyrogens from a variety of sources, e.g. *Proteus vulgaris*, *Salmonella abortus equi* and species of *Pseudomonas* and *Shigella*.

### SUBSTANCES AND PREPARATIONS THAT MUST COMPLY WITH A PYROGEN TEST

#### A. Water for Injection

#### B Infusion Fluids

*Compound Sodium Lactate Injection*

*Dextran Injection*

*Dextrose Injection*

*Strong Dextrose Injection*

*Potassium Chloride Injection*

*Sodium Bicarbonate Injection*

*Sodium Calcium edetate Injection*

*Sodium Chloride and Dextrose Injection*

*Sodium Chloride Injection*

*Sodium Lactate Injection*

#### C. Fermentation products

##### 1 ANTIBIOTICS

*Bacitracin*

*Benethamine penicillin*

*Benzathine penicillin*

*Benzylpenicillin*

- Methicillin Injection  
 Methicillin Sodium.  
 Oxytetracycline Injection.  
 Streptomycin Sulphate  
 Tetracycline  
 Tetracycline Injection  
 Vancomycin Hydrochloride  
 Vancomycin Injection  
 Viomycin Sulphate  
 Viomycin Injection

## 2 ORGANIC ACID SALTS

### Calcium Gluconate Injection

The anticoagulant solutions containing sodium acid citrate that are used in the collection of human blood

## D Animal Products

- Chorionic Gonadotrophin Injection  
 Heparin.  
 Heparin Injection  
 Hyaluronidase  
 Protamine Sulphate

## E Dyes and other substances given intravenously for diagnostic purposes

- Congo Red  
 Indigocarmine  
 Phenolsulphonphthalein  
 Sodium Diatrizoate Injection

## REFERENCES

### Temperature-rise method

- BEWICK, A R M (1960) A review of the occurrence and testing of pyrogens and the production of pyrogen free solutions *Lab Practice*, 9, 153-156  
 D'ARCY, P F and SPURLING, N W (1962) A pyrogen testing unit *Lab Practice*, 11, 458-463  
 DARE, J G (1953) Some observations on the B P and U S P tests for pyrogens, 5, 898-906  
 REPORT (1956) New equipment for pyrogen testing *Lab Practice*, 5, 462-463

- SMITH, K L (1954) Routine pyrogen testing *J Pharm Pharmacol* 6, 309-316  
 WHITTET, T D (1954) A pyrogen testing laboratory *Chem and Drugg* 161, 301  
 WYLIE, D W and TODD, J P (1948) An examination of the sources and the quantitative methods of testing pyrogen *Quart J Pharm* 21, 240-252

### Tolerance

- TENNENT, D M and OTT, W H (1953) Tolerance to bacterial pyrogens in the rabbit *J Amer pharm Ass, Sci Ed* 42, 614-618

### Leucocyte method

- DAWSON, M and TODD, J P (1952) The assay of bacterial pyrogens *J Pharm Pharmacol* 4, 972-978  
 DAWSON, M and TODD, J P (1954) The leucocyte response in the rabbit to pyrogen from *Proteus vulgaris* Part I Mononuclear and temperature responses *ibid* 6, 317-321  
 DAWSON, M, ANDERSON, W and TODD, J P (1954) The leucocyte response in the rabbit to pyrogen from *Proteus vulgaris* Part 2 Neutrophil and temperature responses *ibid* 6, 962-968  
 TODD, J P (1955) Bacterial pyrogens *ibid* 7, 625-641

### Quantitative studies

- DARE, J G (1953) Some quantitative studies on a bacterial pyrogen *J Pharm Pharmacol* 5, 528-546  
 DARE, J G and MOGEY, G A (1954) Rabbit responses to human threshold doses of a bacterial pyrogen *ibid* 6, 325-332  
 OTT, W C (1949) A quantitative assay method for pyrogens *J Amer pharm Ass, Sci Ed* 38, 179-184  
 PERRY, W L M (1954) Standards of pyrogenic activity *J Pharm Pharmacol* 6, 332-338  
 TENNENT, D M and OTT, W C (1952) Pyrogens Assay by the febrile response in rabbits *Analyst* 77, 643

## Appendix 3

### Incompatibilities of Bactericides

INCOMPATIBILITIES have been reported between the following substances and the bactericides under which they are listed. These may not be shown when the concentration of the medicament is low.

**Phenol**  
Aneurine hydrochloride  
Hexamethonium  
Procaine penicillin  
\*Quinine hydrochloride

**Cresol**  
Carbachol  
Ergometrine  
\*Quinine hydrochloride

**Phenylmercuric nitrate**  
Aneurine hydrochloride  
Ascorbic acid  
Ergometrine  
Hexamethonium bromide  
Hexamethonium tartrate  
\*\*Homatropine hydrobromide  
\*\*Hyoscine hydrobromide  
Decamethonium iodide  
Pethidine hydrochloride

**Chlorocresol**  
Benzylpenicillin (on storage)  
Hydrated calcium chloride  
Carbachol  
Chlorphenenamine maleate  
\*Diamorphine hydrochloride  
Ergometrine  
Heparin  
Homatropine hydrobromide  
Mersalyl  
Methadone  
Methyl cellulose  
\*Papaveretum  
\*\*\*Physostigmine salicylate  
\*Quinine hydrochloride  
Sodium morrhuate  
Strophanthin K  
\*Strychnine hydrochloride

Where the substance is marked with one asterisk the incompatibility is shown in the cold, and after

sterilisation. Two asterisks indicate that the incompatibility is shown only in the cold and three that the incompatibility is not shown when sodium metabisulphite is present.

In other cases incompatibility was evident after the normal method of preparation, i.e. in the cold, for antibiotic containing preparations, or post sterilisation, for heat-sterilised injections.

Between phenol and hexamethonium there is therapeutic incompatibility, the ganglionic blocking action of the medicament may be potentiated by the bactericide.

Not every one of these incompatibilities is generally agreed (e.g. that of aneurine with phenylmercuric nitrate). It is possible that some reactions have been due to impurities in the bactericides.

#### REFERENCES

- 1 DAVIS, H (1948) Symposium on modern methods of preservation of medicinal substances and pharmaceutical preparations *Quart J Pharm* 21, 451-454
- 2 HADGRAFT, J W and SHORT, P (1947) The compatibility of chlorocresol *Pharm J* 158, 202, 360
- 3 MCEWAN, J S and MACMORRAN, G H (1947) The compatibility of some bactericides *Pharm J* 158, 260-262
- 4 MIDDLETON, T R and BAVIN, E M (1945) Chlorocresol with ergometrine *Pharm J* 154, 214
- 5 REEDS, D (1947) Compatibility of chlorocresol *Pharm J* 158, 229
- 6 ROBERTS, M (1955) Difficulties associated with the dispensing of phenylmercuric nitrate *Aust J Pharm* 36, 1146 and 1149 (Abstracted in M & B Pharmaceutical Bulletin (1956) 6, 52)

## Appendix 4

### Classification of Injections under Methods of Sterilisation

#### Dry Heat

##### B P INJECTIONS

Deoxy cortone acetate  
Dimercaprol  
Nandrolone phenylpropionate  
Oestradiol benzoate

##### B P C INJECTIONS

Oily phenol

#### Heating in an Autoclave

##### B P INJECTIONS

Adrenaline  
\*Aminophylline  
\*Antimony potassium tartrate  
\*Antimony sodium tartrate  
Apomorphine  
\*Atropine sulphate  
\*Bemegride  
Calcium gluconate  
\*Carbachol  
\*Chloroquine phosphate  
\*Chloroquine sulphate  
Chlorpromazine  
Chlorpheniramine  
\*Cyanocobalamin  
\*Dextran  
\*Dextrose  
Digoxin  
\*Diodone  
\*Dipipanone  
Ergometrine  
Ergotamine

Progesterone  
Testosterone phenylpropionate  
Testosterone propionate

\*Gallamine  
\*Hexamethonium tartrate  
\*Histamine acid phosphate  
\*Hyoscine  
\*Indigo carmine  
Iodipamide methylglucamine  
Lignocaine and adrenaline  
\*Lignocaine hydrochloride  
Meflarsoprol  
Mepyramine  
\*Methadone  
\*Methylamphetamine  
\*Methylene blue  
Methylergometrine  
\*Nalorphine  
\*Neostigmine  
\*Nikethamide  
Pentolinium

\*Pethidine

\*Phenolsulphonphthalim  
Phytomenadione  
\*PicROTOXIN  
Potassium chloride solution (for Potassium chloride Injection)

Procainamide  
Prochlorperazine  
Promethazine hydrochloride  
\*Pyridostigmine  
\*Quinine dihydrochloride

\*Sodium acetrizoate  
\*Sodium bicarbonate

##### B P C INJECTIONS

Bismuth oxychloride  
\*Bismuth sodium tartrate  
\*Strong Dextrose  
Ethanolamine oleate  
\*Leptazol  
\*Mephenesin  
\*Strong solution of Nor-

#### Heating with a Bactericide

##### B P INJECTIONS

\*Diamorphine  
Dimenhydrinate (special method)  
\*Emetine  
\*Mersalyl  
\*Morphine sulphate

\*Sodium calcium edetate

\*Sodium chloride and dextrose  
\*Sodium chloride  
Sodium chromate (<sup>51</sup>Cr)

Sodium diatrizoate  
\*Compound Sodium lactate  
Sodium iodide (<sup>131</sup>I)  
\*Sodium lactate  
Sodium phosphate (<sup>32</sup>P)

\*Sodium stibogluconate  
Stibophen  
Sulphadimidine  
Tubocurarine

adrenaline (for Nor-adrenaline Injection)  
\*Pituitary posterior lobe  
\*Sodium citrate  
\*Sodium nitrite  
Sodium thiosulphate  
\*Strong Solapsone  
Sulphadiazine sodium

Procaine and adrenaline  
\*Sodium aurothiomaleate  
\*Suxamethonium chloride

\* Sterilisation by Filtration is an alternative method.

**B P C INJECTIONS**

\*Ascorbic acid  
\*Morphine and atropine

**Sterilisation by Filtration****B P INJECTIONS**

Aneurine hydrochloride  
Corticotrophin gelatin  
Diphenhydramine  
Heparin  
Hydroxocobalamin  
Insulin  
Menaphthone sodium bisulphite

**B P C INJECTIONS**

Lobeline

**Aseptic Technique****B P INJECTIONS**

†Amylobarbitone  
Benzylpenicillin  
†Chorionic gonadotrophin  
†Corticotrophin  
Corticotrophin zinc hydroxide  
Cortisone

**\*Papaveretum**

Mephentermine  
Oxytocin  
Phentolamine  
Promazine  
Protamune sulphate  
Sulphobromophthalein sodium  
Vasopressin

†Hydrocortisone sodium succinate  
Insulin zinc suspension  
Insulin zinc suspension (Amorphous)  
†Insulin zinc suspension (Crystalline)

Iodised oil, fluid  
Iodised oil, viscous  
Iodophendylate  
Isophane insulin  
Methicillin  
Mustine  
Oxytetracycline  
†Pentamidine  
†Phenobarbitone  
Potassium chloride (final stage)  
Prednisolone trimethylacetate

**B P C INJECTIONS**

Deoxycortone trimethylacetate  
Fortified Procaine penicillin  
Globin zinc insulin  
Human gamma globulin  
Hydrocortisone acetate

Mepacrine methanesulphonate  
†Neoarsphenamine  
†Noradrenaline

Procaine penicillin

Propyliodone  
Propylodone oily  
Protamine zinc insulin  
Quinine dihydrochloride (final stage)  
†Sodium antimonyl-gluconate  
Sodium calciumedetate (final stage)  
Streptomycin sulphate  
†Suramin  
†Suxamethonium bromide  
Tetracycline  
†Thiopentone  
Trimetaphan  
†Tryparsamide  
Vancomycin  
Viomycin

Oxphenarsine hydrochloride  
Tetracycline and procaine

\* Sterilisation by Filtration is an alternative method

† These injections must be prepared *immediately* before use. In some cases, e.g. corticotrophin zinc hydroxide and the depot injections of insulin filtration is used to sterilise the solutions that are aseptically mixed to make the final suspensions

## Appendix 5

### Classification of Injections under Methods of Stabilisation

#### 1. Protection from Light is Necessary

##### *B P Injections*

Adrenaline  
Aneurine hydrochloride  
Chlorpheniramine  
Chlorpromazine  
Chorionic gonadotrophin  
Corticotrophin  
Corticotrophin gelatin  
Cyanocobalamin  
Deoxycortone acetate  
Deoxycortone trimethylacetate  
Diamorphine  
Digoxin  
Diodone  
Diphenhydramine  
Dipranone  
Emetine  
\*Ergometrine  
Ergotamine  
Gallamine  
Histamine acid phosphate  
Human gamma globulin  
Hydroxocobalamin  
Hyoscine  
Iodised oil, fluid  
Iodised oil, viscous  
Iophendylate

Lignocaine and adrenaline  
Menaphthone sodium bisulphite  
Mersalyl  
Methylergometrine  
Morphine sulphate  
Nalorphine  
Nandrolone phenylpropionate  
Neostigmine  
Oestradiol benzoate  
Phentolamine  
Phytomenadione  
PicROTOxin  
Prednisolone trimethylacetate  
Procaine and adrenaline  
Procaine penicillin  
Prochlorperazine  
Progesterone  
Promazine  
Promethazine hydrochloride  
Pyridostigmine  
Quinine dihydrochloride  
Sodium acetrizoate  
Sodium aurothiomaleate

Sodium stibogluconate  
Stibophen  
Sulphadimidine

Testosterone phenylpropionate  
Testosterone propionate

##### *B P C Injections*

Ascorbic acid  
Bismuth oxychloride  
Bismuth sodium tartrate  
Ethanolamine oleate  
Lobelene

Morphine and atropine  
Strong Noradrenaline solution  
Papaveretum  
Sulphadiazine sodium

#### 2. Containers must Comply with the Test for Limit of Alkalinity of Glass

##### *B.P Injections*

Adrenaline  
Aneurine hydrochloride  
Apomorphine  
Atropine sulphate  
Bemegride  
Calcium gluconate  
Corticotrophin  
Corticotrophin gelatin  
Corticotrophin zinc hydroxide  
Cyanocobalamin  
Diamorphine  
Digoxin  
Diodone  
Diphenhydramine  
Dipranone  
Emetine  
Ergometrine  
Ergotamine  
Globin zinc insulin  
Heparin

Histamine acid phosphate  
Hydroxocobalamin  
Hyoscine  
Insulin  
Insulin zinc suspension  
Insulin zinc suspension (Amorphous)  
Insulin zinc suspension (Crystalline)  
Isophane insulin  
Lignocaine and adrenaline  
Mersalyl  
Methylergometrine  
Morphine sulphate  
Nalorphine  
Neostigmine  
Oxytocin  
Phytomenadione  
Potassium chloride

\* Must also be protected from light during the preparation of the injection.

*B P Injections continued*

Procaine and adrenaline	Stibophen	*Hydroxocobalamin	4.0-5.5
Protamine zinc insulin	Suxamethonium chloride	*Insulin	3.0-3.5
Pyridostigmine	Tubocurarine	*Globin zinc insulin	3.0-3.5
Quinine dihydrochloride	Vasopressin	*Isophane insulin	7.1-7.4
Sodium calciumedetate		*Protamine zinc insulin	6.9-7.3

*B P C Injections*

Ascorbic acid	Morphine and atropine	*Insulin zinc suspension (amorphous)	7.2-7.5
Bismuth sodium tartrate	Strong Noradrenaline solution	*Insulin zinc suspension (crystalline)	7.2-7.5
Dextrose, strong	Papaveretum	Iodipamide methylglucamine	7.0-7.6
Leptazol	Pituitary posterior lobe	Lignocaine and adrenaline	3.0-4.5
Lobeline		Mephentermine	4.0-6.5
Mephenesin		Mepyramine	4.5-5.5
		*Mersalyl	7.6-8.2
		*Methylergometrine	2.9-3.5
		*Nalorphine	2.7-3.3
		Oxytetracycline	2.0-3.0

## 3 The pH must lie within Specified Limits

*B P Injections*

Adrenaline	3.2-3.6	Oxytocin	3.0-4.0
Aminophylline	9.2-9.6	Pentolinium	6.0-7.0
Aneurine hydrochloride	2.8-3.4	Phentolamine	3.8-5.0
Apomorphine	3.0-4.0	Phytomenadione	4.8-6.0
Benzylpenicillin	5.5-7.5	Prednisolone trimethylacetate	6.0-7.0
	(10% w/v solution)	Procainamide	4.5-5.5
Chloroquine phosphate	3.5-4.5	Prochlorperazine	5.5-6.5
Chloroquine tartrate	4.0-5.5	Promazine	4.4-5.2
Chlorpheniramine	4.0-5.2	Promezathine hydrochloride	5.0-6.0
Chlorpromazine	5.0-6.5	Propyldone	6.5-7.5
Chorionic gonadotrophin	6.0-8.0	Pyridostigmine	5.7-6.3
	(1% w/v solution)	Quinine dihydrochloride	1.5-3.0
Corticotrophin	3.0-5.0	Sodium acetizotate	6.5-8.5
Corticotrophin gelatin	4.5-7.0	Sodium calciumedetate	6.5-8.0
*Corticotrophin zinc hydroxide	7.6-8.1	Sodium chloride and dextrose	3.5-6.5
*Cyanocobalamin	4.0-5.5	Sodium chromate ( <sup>51</sup> Cr)	5.0-8.0
Deoxycortone trimethylacetate	6.0-7.0	Sodium iodide ( <sup>131</sup> I)	7.0-8.0
Dextran	5.0-7.0	*Sodium lactate	5.0-7.0
Dextrose	3.5-6.5	*Compound sodium lactate	5.0-7.0
Digoxin	6.7-7.3	Sodium phosphate ( <sup>32</sup> P)	6.0-7.0
Dihydrhydrinate	6.8-7.2	Sodium stibogluconate	5.0-6.0
	6.8-7.0	Stibophen	5.0-5.5
*Dimercaprol	{(at adjustment) 5.5-6.5 (finally)}	Streptomycin sulphate	5.0-6.5
		Sulphadimidine	10.0-11.0
Diiodone	6.0-8.0	Suxamethonium chloride	3.0-5.0
Diphenhydramine	5.0-6.0	Tetracycline	2.0-3.0
*Dipipanone	4.0-5.6		(10% w/v solution)
*Emetine	2.7-4.0	Tubocurarine	4.0-6.0
*Ergometrine	2.7-3.5	Vasopressin	3.0-4.0
*Ergotamine	3.1-3.7		
Gallamine	5.5-7.5		
*Heparin	7.0-8.5		
*Hexamethonium tartrate	6.0-8.0		
Hydrocortisone sodium succinate	7.0-8.0		
	(6.7% w/v soln.)		

\* Preparation involves pH adjustment

**4 A Buffering Agent must or may be Added**

Where the buffer is specified in the official book it is stated opposite the name of the injection

**B.P. Injections**

Benzylpenicillin	
Chlorpromazine	
Cyanocobalamin	
*Deoxycortone trimethyl acetate	
*Digoxin	—citric acid and sodium phosphate
*Hydrocortisone sodium succinate	
Hydroxocobalamin	
*Insulin zinc suspension	—sodium acetate
*Insulin zinc suspension (amorphous)	—sodium acetate
*Insulin zinc suspension (crystalline)	—sodium acetate
*Isophane insulin	—sodium phosphate
*Mephenetermine	
*Oxytetracycline	
*Prednisolone trimethylacetate	
*Procaine penicillin	
Fortified procaine benzyl penicillin	
*Prochlorperazine	
*Promazine	
Propylthiouracil	
*Protamine zinc insulin	—sodium phosphate
Sodium acetrizoate	
Sodium antimonylgluconate	
*Stibophen	—sodium acid phosphate
Streptomycin sulphate	
Suxamethonium chloride	
*Tetracycline	
<b>B.P.C. Injections</b>	
*Leptazol	—sodium phosphate
*Oxphenarsine	
*Tetracycline and procaine	

**5 Air is replaced by Nitrogen or other Suitable Gas****B.P. Injections**

Apomorphine	Ergotamine (and the dry solid)
Chlorpheniramine	Methylergometrine (and the dry solid)
Chlorpromazine	
Dimercaprol	Phentolamine

\* Addition is obligatory

Prochlorperazine	
Promazine	
Promezathine hydrochloride	

**B.P.C. Injections**

Ascorbic acid	
Sodium thiosulphate	Sulphadiazine sodium

**6 Contact with Metals must be avoided****B.P. Injections**

Aminophylline	
Diodone	Mersalyl

**B.P.C. Injections**

Ascorbic acid	
	Leptazol

**7 A Reducing Agent is Added****(a) SODIUM METABISULPHITE 0.1 PER CENT****B.P. Injections**

Adrenaline	
Apomorphine	Menaphthone sodium bisulphite (0.2 per cent)
Diamorphine	Morphine sulphate
Lignocaine and adrenaline	Phentolamine
	Procainamide
	Procaine and adrenaline

**B.P.C. Injections**

Morphine and atropine	
	Strong solution of noradrenaline

**(b) SODIUM SULPHITE (the equivalent of 0.2 per cent Na<sub>2</sub>SO<sub>3</sub>, the crystalline salt has 7 H<sub>2</sub>O)****B.P. Injections**

Gallamine	
	(c) DEXTROSE

**B.P. Injections**

Carbachol	
	Phentolamine

**8 The Solvent must be Water for Injection free from Carbon Dioxide****B.P. Injections**

Aminophylline	
Amylobarbitone	Phenobarbitone

**B.P.C. Injections**

Sulphadiazine sodium	
----------------------	--

**9 The Solvent must be Water for Injection free from Dissolved Air**

*B.P. Injections*

Apomorphine	Prochlorperazine
Chlorpheniramine	Promazine
Chlorpromazine	Promezathine hydrochloride
Ergometrine	Sulphadimidine
Ergotamine	Tubocurarine
Methylergometrine	

**10 The Injection must be packed in Ampoules**

The reason is given against each injection

*B.P. Injections*

Aminophylline—the solvent is Water for Injection free from Carbon Dioxide

Apomorphine—the solvent is Water for Injection free from Dissolved Air and the air is replaced by an inert gas

Calcium gluconate—particles from rubber closures might cause crystallisation of the supersaturated solution

Digoxin—partially volatile solvent

Dimercaprol—the air is replaced by an inert gas

Diodone—avoidance of metal contact is necessary

Ergometrine—the solvent is Water for Injection free from Dissolved Air and the air is replaced by an inert gas

Ergotamine—the solvent is Water for Injection free from Dissolved Air and the air is replaced by an inert gas

Histamine acid phosphate—decomposes on exposure to air

Iodophendylate—deteriorates when exposed to air and light

Menaphthone sodium bisulphite—large volumes may be given by intravenous injection in hypoprothrombinæmia, therefore necessary to omit a bactericide

Mersalyl—avoidance of metal contact is necessary

Methylergometrine—the solvent is Water for Injection free from Dissolved Air and the air is replaced by an inert gas

Nikethamide—attacks rubber

Promazine—the solvent is Water for Injection free from Dissolved Air and the air is replaced by an inert gas

Sulphadimidine—the air is replaced by an inert gas

Tubocurarine—the air is replaced by an inert gas

*B.P.C. Injections*

Ascorbic acid—the air is replaced by an inert gas

Lobeline—a bactericide is not permitted

Sodium nitrite—large volumes may be given by intravenous injection in cyanide poisoning, therefore necessary to omit a bactericide

Sodium thiosulphate—the air is replaced by nitrogen

Sulphadiazine—the air is replaced by an inert gas

## Appendix 6

### Latin Terms used in Prescriptions

**PRESCRIPTION** (*praescriptio—præ, before, and scribo, I write*) is the term applied to the formula and directions written by a physician for the preparation and use of remedies.

The details of a prescription are written in the following order—

- 1 The superscription or heading, which consists of the sign, R\* an abbreviation for *recipe*, take
- 2 The inscription, which designates—
  - (a) The ingredients
  - (b) The quantity of each required
- 3 The subscription, which gives directions to the dispenser as to—
  - (a) The form of the remedy
  - (b) Instructions relating to its preparation
  - (c) Quantity to be sent and manner of sending
- 4 The signature (from *signetur*,† let it be labelled), consisting of directions to the patient (*Eger (m)*, *Agra (f)*). This part of the prescription declares
  - (a) The method of administration or application
  - (b) The dose (if the remedy is for internal use)
  - (c) The time of administration or application
  - (d) The vehicle of administration or the means of application

\* Although usually written as shown, it could be *Rj* the originally being an invocation to Jove (Jupiter), the god of healing.

† D S may be found at the beginning of the Signature in old prescriptions. This is an abbreviation for *Da, Signa, Gice and label* or *Datur Signetur, Let it be given and labelled*.

- (e) The part of the body to which the remedy is to be applied (if for external use)
- 5 The patient's name
- 6 The prescriber's name or initials and the date

#### TYPICAL PRESCRIPTION

1 Superscription	Recipe
2 Inscription	Potassii Bromidi      3 ij Syrupi Aurantii      3 j Aquam Menthae Piperite      ad 5 vj
3 Subscription	Fiat mistura
4 Signature	Signetur— Cochlearie magnum ter in die ex aqua sumen- dum
5 Patient's Name	For Mrs Brown
6 Prescriber's Initials	W R
7 The Date	20 February 1965

The above order forms a convenient basis for learning the Latin terms commonly used in prescriptions, and has been adopted for this reason. As mentioned (p. 3), the student should thoroughly learn two or three pages each week. The meaning of the Latin terms used in the subscription and signature accompanying the exercises in the chapters on Mixtures and Emulsions, are given immediately below to assist the student in course of learning the full range of Latin terms. This part closely follows the exercises, but additional expressions are included to save needless repetition.

#### EXERCISE 4.1

*Ad*

means 'up to' or 'sufficient to produce,' and must not be confused with the English word 'add.'

*Flat mistura*

means 'make a mixture' (literally 'let a mixture be made'). In labelling, the preparation is named from the subscription, in this case the label is headed 'The Mixture.'

- Signetur* means 'label' (literally 'let it be labelled') The Latin word *Signa* is an alternative
- Cochleare* means 'spoonful,' and is therefore indefinite unless accompanied by an adjective The adjectives (placed after the noun) used to qualify it are—

<i>amplum</i>	any of which, coupled with <i>cochleare</i> , means 'one tablespoonful'
<i>magnum</i>	In Latin, the word 'one' is not expressed, being implied in the singular form of the noun <i>cochleare</i> . The indefinite article 'a' is not sufficiently precise, and 'one' should always be used in translating these directions into English
<i>maximum</i>	
<i>plenum</i>	
<i>medium</i>	either of which, coupled with <i>cochleare</i> , means 'one dessertspoonful'
<i>modicum</i>	
<i>minimum</i>	either of which, coupled with <i>cochleare</i> , means 'one teaspoonful'
<i>parum</i>	

Cardinals are added when more than 'one' is indicated  
For the present these will be only—

*duo* (neuter form), 'two'  
*tria* (neuter form), 'three'

*Cochleare* changes to *cochlearia* in the plural, and the ending *-um* in the adjectives *amplum*, etc., becomes *-a*, thus—

*cochlearia ampla duo* = two tablespoonfuls

*ter die* means 'three times a day' *Ter* is an adverbial numeral, and others which should be noted now are *semel* (once), *bis* (twice), and *quater* (four times)

*post cibos* means 'after meals' The singular form *post cibum* (lit 'after food') is an alternative, and should also be translated 'after meals' Similarly, *ante cibos* and *ante cibum* mean 'before meals,' while *inter cibos* (*cibum*) means 'between meals'

*sumendum* means 'to be taken' An alternative is *capiendum*, which, like *sumendum*, is a gerundive, changing the final syllable *-um* when necessary, to agree with the noun to which it refers

The gerundive is usually placed at the end of the signature, but, in translating, it is placed in the usual English sequence, thus, the directions for Exercise 1 read 'One tablespoonful to be taken three times a day after meals'

#### EXERCISE 4.2

Note carefully that the direction 'to be taken three times a day' applies to the quantity of medicine prescribed above it, i.e. to  $\frac{1}{2}$  oz Many prescribers repeat the quantity thus  $\frac{1}{2}$  ss *ter in die capienda*, but this is quite unnecessary Quantities such as  $\frac{1}{4}$  oz,  $\frac{1}{2}$  oz, must not appear on labels giving directions to a patient—they might have no immediate significance, or be interpreted wrongly

(continued overleaf)

The accepted interpretations are—

- 1 drachm (3 i) = One teaspoonful
- 2 drachms (3 ii) = Two teaspoons or one dessertspoonful
- $\frac{1}{2}$  oz (3 iv or 3 ss) = One tablespoonful
- 1 oz (3 i) = Two tablespoonfuls

*Mitte* means 'send'

---

#### EXERCISE 4.4

*semihora* means 'half an hour'  
*ante jentaculum* means 'before breakfast'

---

#### EXERCISE 4.4

See the note above under Exercise 4.2—the dose to be taken is the quantity of medicine prescribed above the signature, i.e. 15 ml. Metric quantities are interpreted as follows—

- 4 ml = one teaspoonful
  - 8 ml = two teaspoons or one dessertspoonful
  - 15 ml = one tablespoonful
  - 30 ml = two tablespoonfuls
- 

#### EXERCISE 4.5

See the note above under Exercise 4.2—the dose to be taken is the quantity of medicine prescribed above the signature, i.e. 1 oz = two tablespoonfuls

---

#### EXERCISE 4.6

*secundis horis* means 'every two hours.' Alternative expressions are—

*quaque (or omni) secunda hora* } 'every two hours'  
*alternis horis* } the usual translation  
*quaque (or omni) alterna hora* } for all these

Other 'hour time' expressions which should be noted now are—

<i>omni hora</i>	} = every hour
<i>quaque (or omni) singula hora</i>	
<i>singulis horis</i>	} = every three hours
<i>quaque (or omni) tertia hora</i>	
<i>tertius horis</i>	} = every four hours
<i>quaque (or omni) quarta hora</i>	
<i>quartus horis</i>	} = every six hours
<i>quaque (or omni) sexta hora</i>	
<i>sextus horis</i>	
<i>doses sex</i>	means 'six doses.' The cardinals (p. 572) up to 12 may now be learnt to advantage

---

<b>EXERCISE 4.7</b>	<i>ex lacte</i>	means 'in milk' or 'with milk' Another term to be noted now is—
	<i>dolore urgente</i>	<i>ex aqua</i> , meaning 'in water' or 'with water' means 'when the pain is severe'. <i>Urgente</i> carries a meaning appropriate to the condition to which it refers, thus <i>tussi urgente</i> is translated 'when the cough is troublesome'
<b>EXERCISE 5.2</b>	<i>Fiat haustus</i>	means 'make a draught' As mentioned earlier, the preparation is named from the subscription, in this case 'The Draught'. The fact that it is an emulsion is disregarded in favour of the name indicated in the subscription
	<i>statim</i>	means 'at once' or 'immediately' an alternative is <i>illico</i>
<b>EXERCISE 5.3</b>	<i>emulsio</i>	means 'emulsion,' and the preparation is therefore labelled 'The Emulsion' An alternative is <i>emulsum</i>
<b>EXERCISE 5.5</b>	<i>more dicto</i> <i>danda</i>	means 'as directed' <i>Modo dicto</i> is an alternative means 'to be given' The preparation is for an infant, and 'to be given' is therefore more appropriate than 'to be taken'
<b>EXERCISE 9.15</b>	<i>Fiat linimentum</i>	means 'make a liniment,' and the preparation is therefore labelled 'The Liniment'
<b>EXERCISE 9.11</b>	<i>utenda</i>	means 'to be used' An alternative is <i>exhibenda</i> , although the latter is sometimes used, meaning 'to be taken', the choice depends on the nature of the preparation
<b>EXERCISE 5.14</b>	<i>quotidie</i>	means 'daily' An alternative is <i>indies</i>
<b>EXERCISE 5.18</b>	<i>secundum artem</i>	means 'pharmaceutically,' or 'in a pharmaceutical manner,' or 'following pharmaceutical usage or custom' (literally 'according to art')
	<i>enema</i>	means 'enema' or 'rectal enema'
	<i>hora somni</i>	means 'at bedtime,' literally 'at the hour of sleep' An alternative is <i>hora decubitus</i> , literally 'at the hour of lying down'

## LATIN TERMS AND ABBREVIATIONS COMMONLY USED IN PRESCRIPTIONS\*

### TERMS RELATING TO THE INSCRIPTION

Standard Weights and Measures with their Latin equivalents and abbreviations are shown on pp 11-13.

In addition to fixed quantities, the following are used—

<i>Term or Phrase</i>	<i>Abbreviation</i>	<i>Meaning</i>	<i>Latin Name</i>	<i>Abbreviation</i>	<i>English Name</i>
Quantum sufficiat			Haustus	ht	A draught
Quantum sufficit	q.s.	As much as is sufficient	Inhalatio	inhal	An inhalation
Quantitatem sufficientem			Injectio	inj	An injection
Ad		Up to, sufficient to produce	Injectio	inj	A hypodermic injection
Ana	āā, aa	Of each	hypodermica	hyp	
Partes æquales	pt. æq	Equal parts	Insufflatio	insuff	An insufflation
			Linctus	linct.	A linctus
			Linimentum	lin	A liniment
			Lotion	lot	A lotion
			Mistura	m, mist	A mixture
			Nebula	neb	A spray solution
			Oblatum	oblat	A cachet
			Pasta	past	A paste
			Pastillus	pastill	A pastille
			Pessus	pess	A pessary
			Pigmentum	pigm	A paint
			Pilula	pil.	A pill
			Pulvis	pulv	A powder
			Pulvis	pulv	A dusting powder
			conspersus	conspers	
			Sternutamentum	sternut	A snuff
			Suppositorium	suppos	A suppository
			Tabella	tab	A tablet
			Tabletta	tab	A tablet
			Trochuscus	troch	A lozenge
			Unguentum	ung	An ointment
			Nomen Proprium	N P	Proper name (i.e. label with name of article)

### TERMS RELATING TO THE SUBSCRIPTION

#### Forms of Remedies

<i>Latin Name</i>	<i>Abbreviation</i>	<i>English Name</i>
Aurinarium	aurin.	An ear cone
Auristilla	auristill	The ear drops
Buginarium	buginar	A nasal bougie
Capsula	caps	A capsule
Capsula amyacea	caps amyac	A cachet
Capsula gelatina	caps gelat	A gelatin capsule
Capsula vitrea	caps vitrea	A glass capsule
Cataplasmata	cataplasma	A poultice
Cereolus	cereol	An urethral bougie
Charta	chart	A powder
Collunarium	collun.	A nose wash
Collutonum	collut.	A mouth wash
Collyrium	collyr	An eye lotion
Confectio	conf	A confection
Cremor	crem	A cream
Depilatorium	depilat	A depilatory
Emplastrum	emp	A plaster
Emulsio	emul	An emulsion
Enema	—	An enema
Gargarisma	garg	A gargle
Gelatina	gelat	A jelly
Guttae	gtt	Drops

#### Instructions Relating to its Preparation

##### (a) General Terms

<i>Term or Phrase</i>	<i>Abbreviation</i>	<i>Meaning</i>
Addit., Addatur	add (or ad)	Add Let (it) be added
Calefac, Calefiat	calef	Warm Let (it) be warmed
Fiat	ft	Let (it) be made
Fiant	ft	Let (them) be made
Misce, Misceatur	m	Mix Let (it) be mixed
Misce fiat	m ft m	Mix to make a mixture
mixtura	m	Mix pharmaceutically (it according to art)
Misce secundum artem	m s a	Dissolve
Solve	—	

\* A full vocabulary of terms in alphabetical order appears in *Latin for Pharmaceutical Students* (Cooper and McLaren).

## (b) Relating to Powders

Term or Phrase	Abbreviation	Meaning	Term or Phrase	Abbreviation	Meaning
Divide	div	Divide Let (it) be divided	Capiantur	cap	Let (them) be taken
Dividatur			Capiendus	capiend	To be taken
Dividatur in partes aequales	div in pt æq	Divide into equal parts	Da	d	Give
Fiat pulvis subtilis	ft pulv subtil	Make a fine powder	Dandus	dand	To be given
Tere Teratur	ter	Rub Let (it) be rubbed	Degluviendus	deglut	To be swallowed
Tere bene simul	ter bene simul	Rub well together	Detur	d	Let (it) be given
			Dentur	d	Let (them) be given
			Infricandus	infricand	To be rubbed in
			Infricetur	infric	Let (it) be rubbed in

## (c) Relating to Pills

Term or Phrase	Abbreviation	Meaning			
Fiant pilule due	ft pil ii	Make two pills	Inhaletur	inhal	Let (it) be inhaled
Involve	involv	Roll To be rolled	Inspiretur	inspir	Let (it) be inspired
Volvendus	volvend		Instillandus	instilland	To be dropped in
Saccharatæ	sacch	Sugared	Instilletur	instill	Let (it) be dropped in
Tunicatæ	tunicat	Sugar-coated	Instillentur	instill	Let (them) be dropped in
		Coated (meaning varnished)	Miscendus	miscend	To be mixed
Tunicetur	tunic	Let (it) be varnished	Sugatur	sugatur	Let (it) be sucked
Tunicentur	tunic	Let (them) be varnished	Sugendus	sugend	To be sucked
			Sumat	sum	Let (sum) take
			Sumatur	sum	Let (it) be taken
			Sumantur	sum	Let (them) be taken

## Quantity to be Sent and Manner of Sending

Term or Phrase	Abbreviation	Meaning			
Duplum	duplum	Twice the quantity	Sumendus	s or sum	To be taken
In phiala	—	In a bottle	Ut antea	u a	As before
Mitte	mitt	Send	Utendus	u or utend	To be used
Phiala prius agitata	P p a	The bottle being first shaken (i.e attach a Shake the Bottle label)	Dose		
			FOR FLUIDS		

## (a) Expressed in Words

Term or Phrase	Abbreviation	Meaning			
Cochleare	{ amplum magnum maximum plenum	coch	{ amp mag max plen		
Cochleare	{ medium modicum	coch	{ med mod		
Cochleare	{ minimum parvum	coch	{ min parv		
Cochlearia magna duo*	coch mag ii				
Cochlearia parva tria *	coch parv ii				
Dimidium	dimid				
Reliquum	reliq				

## TERMS RELATING TO THE SIGNATURE

## Method of Administration or Application

Term or Phrase	Abbreviation	Meaning			
Addendus	addend	To be added			
Applicandus	applicand	To be applied			
Applicat	—	Let (hum) apply			
Applicetur	applicat	Let (it) be applied			
Capiat	cap	Let (hum) take			
Capiatur	cap	Let (it) be taken			

\* For numerals see p. 572

## (b) Expressed by Symbols

Term or Phrase	Symbol	Meaning	Term or Phrase	Abbreviation	Meaning
—	ʒ j	One teaspoonful	Ter quotidie	ter quot.	Three times daily
—	ʒ ij	Two teaspoonfuls	Vices	vic.	Time, times
—	ʒ iv	One dessertspoonful	Ad secundum vicem	ad 2nd vic	To the second time
—	ʒ fs	One tablespoonful	Ad tres vices	ad 3 vic	For three times
—	ʒ J	Two tablespoonfuls			

## (c) Expressed in the Metric System

Term or Phrase	Abbreviation	Meaning
—	4 ml	One teaspoonful
—	8 ml.	Two teaspoonfuls
—	15 ml.	One dessertspoonful
—	30 ml.	Two tablespoonfuls

## Other Terms

Term or Phrase	Abbreviation	Meaning
Ad libitum	ad. lib	As much as you please
Dosis	dos	A dose
Gradatum	grad	Gradually
Guttatum	guttatum	Drop by drop
Mensura	mens	By measure
Pro	pro	For On behalf of
Pro dosi	—	As a dose

## Time of Administration or Application

## (a) Times per Day

Term or Phrase	Abbreviation	Meaning
Semel* in die	sem. in die	Once a day
die	sem die	Twice a day
Bis in die	b i d	Three times a day
Bis die	b d	Four times a day
Ter in die	t.i.d.	Six times a day
Ter die	sex. d.	Or
Quater in die	q i d	Two or three times a day
die	q d	Three or four times a day
Sexies in die	sex. i. d.	Five or six times a day
Vel. Ve (enclitic)	—	Daily
Bis terve in die	b t i d.	Daily
Ter quaterve die	t. q d	Daily
Quinques vel sexies in die	quin. vel sex. i. d.	Daily
Indies	indies	Daily
Quotidie	quot.	Daily

\* Notice adverbial numerals

Term or Phrase	Abbreviation	Meaning
Prima luce	prim. luc	Early in the morning
Primo mane	prim. m.	Early in the morning
Mane	m	In the morning
Omn. mane	o m	Every morning
Jentaculum	jentac	Breakfast
Meridie	—	Noon
Prandium	prand.	Dinner
Vespere	vesp	In the evening
Nocte	n.	At night
Inter noctem	inter noct.	During the night
Omn. nocte	o n	Every night
Hora decubitus	h d	At bedtime
Hora somni	h.s.	At bedtime
Nocte et mane	n. et m	Night and morning
Nocte manequae	n.m	Night and morning
Hac nocte	hac noct.	To-night
Cras vespera	cras vesp	To-morrow evening
Mane sequenti	m seq	The following morning

## (b) Particular Parts of the Day

Term or Phrase	Abbreviation	Meaning
Die Soli	—	Sunday
Die Lunæ	—	Monday
Die Martis	—	Tuesday
Die Mercurii	—	Wednesday
Die Iovis	—	Thursday
Die Veners	—	Friday
Die Saturni	—	Saturday
Hodie	—	To-day
Cras	—	To-morrow
Hebdomada	hebdom	A week
Septimana	—	A week
Ter in hebdomada	t. in hebdom	Three times a week

## (c) Days of the Week and Related Terms

Term or Phrase	Abbreviation	Meaning
Die Soli	—	Sunday
Die Lunæ	—	Monday
Die Martis	—	Tuesday
Die Mercurii	—	Wednesday
Die Iovis	—	Thursday
Die Veners	—	Friday
Die Saturni	—	Saturday
Hodie	—	To-day
Cras	—	To-morrow
Hebdomada	hebdom	A week
Septimana	—	A week
Ter in hebdomada	t. in hebdom	Three times a week

## (d) 'Hour' Time

Term or Phrase	Abbreviation	Meaning
Omn. hora. Quaque hora	o h. qq. h.	Every hour

Term or Phrase	Abbreviation	Meaning	Term or Phrase	Abbreviation	Meaning
Omni singula hora	o s h qq	Every hour	Frequentius	—	More frequently
Quaque singula hora	s h		Frequentissime	—	Very frequently
Omni secunda hora	o sec h	Every second hour	Lente	—	Slowly
Quaque secunda hora	qq sec h		More dicto	m.d	{As directed
Omni alterna hora	o alt h	Every alternate hour	Modo dicto	m d	
Quaque alterna hora	qq alt h		Pro re nata	p r n	Occasionally
Omni tertia hora	o tert h	Every third hour	Quoties opus sit	quot o s	As often as necessary
Quaque tertia hora	qq tert h		Sæpe	—	Often
Omni quarta hora	o q h	Every fourth hour	Sæpius	—	More often
Quaque quarta hora	qq q h		Sæpissime	—	Very often
Omni sexta hora	o sext h	Every sixth hour	Si dolor urgeat	si dol urg	If the pain is severe
Quaque sexta hora	qq sext h		Si opus sit	s o s	When required
Singulis horis	sing hor	Every hour	Statim	stat	When necessary
Secundis horis	sec hor	Every two hours	Tussi urgente	tuss urg	Immediately At once
Altermis horis	alt hor	Every two hours			{When the cough is troublesome
Tertius horis	tert hor	Every three hours			If the cough is troublesome
Quartus horis	quart hor	Every four hours			
Sextus horis	sext hor	Every six hours			

#### The Vehicle of Administration or the Means of Application

Term or Phrase	Abbreviation	Meaning	Term or Phrase	Abbreviation	Meaning
Cum	c c	With	Cum parte æquale	c pt æq	With an equal quantity
Cum duplo	c dup	With twice as much	Cum penicillo	c pen	With a camel hair brush
Cum tanto	c tant	With as much	Cyathus	cyath	A glass
Cyathus	cyath		Cyathus amplius	cyath amp	A tumbler
Cyathus magnus	cyath. mag		Cyathus vinarus	cyath vin	A wineglass
Cyathus vinosus	cyath vin		Cyathus vinosus	cyath vin	A wineglass
E	—	{With	Ex	—	Out of
Ex lacte	e lact		Ex vino	e vin	With milk
Ex vino	e vin		Ex aqua	ex aq	With wine
Ex aqua	ex aq		Ex aquæ semicyatho	ex aq semi	With water
			magno	cyath mag	With half a tumblingful of water
Dolore urgente	dol urg	When the pain is severe	Ligamentum	ligament	Bandage
Frequenter	freq	Frequently	Ope penicilli	ope pen	By means of a camel hair brush
			Paulum	paul	A little
			Paululum	paul	A little
			Pauxillum	paux	A little

† Notice ordinals

Term or Phrase	Abbreviation	Meaning	Term or Phrase	Abbreviation	Meaning
E paullo E paulullo E pauxillo	aquæ	With a little	Gutturi	gutt	To the throat
	bullentis	bull boiling water	In aures sinistram	in aur sinist	Into the left ear
	aquaæ	aq With a little	In oculum dextrum	in ocul dext	Into the right eye
	serventis	serv boiling water	In singulas aures	in sing aur	Into each ear
	aquaæ	aq With a little	Jugulo	jug	To the throat
	e paul	e paul	Naso	—	To the nose
	calidæ	e paul	Oculis	ocul	For the eyes
	aquaæ	e paux	Oculo	ocul	To the eye For the eye
	tepidæ	tep	Pro capillis	pro capill	For the hair
	aquaæ	aq With a little	Pro oculis	pro ocul	For the eyes
Poculus	gelidæ	gel cold water	Pro oculo levæ	pro ocul levæ	For the left eye
	aquaæ	aq With a little	Pro singulis oculis	pro sing ocul	For each eye
	communis	com tap water			
	poc	A cup			
Sindon	—	Cambrie			
Super gossypium	sup gossyp	Upon cotton wool			
Super linteum	sup lint	Upon lint			

## The Part of the Body to which to be Applied

## (a) General Terms

Term or Phrase	Abbreviation	Meaning
Dexter	dext	Right
Dolenti partu	dolent part	To the afflicted part
Dolentibus partu	dolent part.	To the afflicted parts
bus	—	Left
Lævus	læv	Left
Parti affectæ	p a	To the affected part
Parti affectæ applicandus	p a a	To be applied to the affected part
Partibus affectis	p a	To the affected parts
Sinister	sinist	Left

## (b) Terms Relating to the Body

Term or Phrase	Abbreviation	Meaning
Brachio	brach	To the arm
Capiti	capit	To the head
Corpon	corp	To the body
Cruri	—	To the leg
Mamma	—	The breast
Pector	pect	To the chest
Sterno	stern	To the chest
Thoraci	thorac	To the chest

## (c) Terms Relating to the Head

Term or Phrase	Abbreviation	Meaning
Auri	auri	To the ear For the ear
Auribus	auribus	To the ears For the ears

## NUMERALS

The cardinals refer to number and, therefore, are translated, one, two, three, four, etc

The ordinals refer to position and, therefore are translated, first, second, third, fourth, etc

The adverbs qualify verbs and, therefore, are translated, once, twice, three times, four times, etc

Arabic Roman No.	Symbols	Cardinals	Ordinals	Adverbs
1	I unus	primus, a -us	semel (once)	
2	II duo	secundus or alter	bis (twice)	
3	III tres, tria (n.)	ter (three times)		
4	IV quatuor or quartuor	quartus	quater (four times)	
5	V quinque	quintus	quinquies	
6	VI sex	sexus	sexes	
7	VII septem	septimus	septies	
8	VIII octo	octavus	octies	
9	X decem	decimus	decies	
10	XI undecim	undecimus	undecies	
11	XII duodecim	duodecimus	duodecies	
12	XIII tredecim	tredecimus	tredecies	
13	XIV quatuordecim	quattuordecimus	quatuor decies	
15	XV quindecim	quinquagesimus	quinquages	
16	XVI sedecim	sexagesimus	sexages	
17	XVII septendecim	septagesimus	septages	
18	XVIII duodecim	duodecagesimus	duodecages	
19	XIX undeviginti	undevigesimus	undeviges	
20	XIX viginti	vicesimus	vicies	
21	XXI et vicesimi	primus et vicesimus, or vicesimus primus	secundus et vicesimus	
22	XXII duo et viginti	or viginti duo	alter et vicesimus	
23	XXIII tres et viginti	or viginti tres	tertius et vicesimus	
28	XXVIII duodecimtriginta	duodecimtriginta	duodecimtrigintas	
29	XXIX undeviginti	undeviginti	undeviginties	
30	XXX triginta	triginta	triges	
40	XL quadragesita	quadragesita	quadrages	
50	L quinquaginta	quinquaginta	quinquages	
60	LX sexagesita	sexagesita	sexages	
70	LXX septagesita	septagesita	septages	
80	LXXX octagesita	octagesita	octages	
90	XC nonagesita	nonagesita	nonages	
100	C centum	centum	centes	
200	CC ducenti, &c. &c.	ducentes	ducenties	
300	CCC trecenti, &c. &c.	trecentes	trecenties	
400	CCCC quadragesenti, &c. &c.	quadragesentes	quadragesenties	
500	D or I quingentis, &c. &c.	quingentes	quingentes	
600	DC sexcenti, &c. &c.	sexcentes	sexcenties	
700	DCC septingentis, &c. &c.	septingentes	septingentes	
800	DCCC octingentis, &c. &c.	octingentes	octingentes	
900	DCCCC nonagesenti, &c. &c.	nonagesentes	nonagesentes	
1000	M or CI milie	milie	milies	
2000	MV du milles	du milles	du milles	



## Appendix 7

### Posology

**Posology** (derived from Greek *posos*, how much, and *logos*, science) is the branch of medicine dealing with Doses. These cannot be rigidly fixed, as, among other things, allowance must always be made for the age and condition of the patient, and the severity of the disease.

Other factors influencing dosage are—

**Natural Tolerance** This is well illustrated in children, who tolerate comparatively large doses of arsenic, belladonna, and calomel.

**Acquired Tolerance** This is illustrated by drug addicts, who have over a long period acquired the ability to take, with comparative safety, large doses of certain drugs, which, in normal persons, would produce harmful or fatal effects. Morphine, heroin, and cocaine are examples of drugs to which tolerance can be acquired.

**Idiosyncrasy** This term is used to indicate exceptional and individual intolerance towards certain drugs. For example, although very large doses of quinine are tolerated by some people, there are others with whom comparatively small doses give rise to unpleasant symptoms of a temporary nature, which are collectively called 'quinism'.

**Route by which the Dose is Administered** The rate of absorption of a drug, and consequently the rapidity with which its effect is produced, varies according to the method of administration. In general, the rapidity of absorption increases with administration in the following order—

Oral → Subcutaneous → Intramuscular → Intravenous. Hence the dose of many substances by injection is less than the oral dose, example, Methylergometrine Maleate.

**Frequency** Oral doses given in the *Pharmacopœia* may be repeated three or four times in twenty-four

hours if no frequency is specified (B.P. 1963 General Notices).

**Degree of Absorption** The constituents of different preparations of the same drug are not always absorbed to the same extent. Consequently the maximum dose of one preparation may represent a larger amount of active constituent than another.

The B.P. gives a minimum and a maximum dose, representing the average dose usually prescribed for adults. The official doses are not binding upon a prescriber, and, considering the above factors, it is apparent that doses in excess of the official maxima may be required in specific cases (Hurst, 1935). These are usually underlined or initialled by the prescriber, but, failing this, it is the duty of the pharmacist to satisfy himself that the overdose has not been prescribed inadvertently.

It is not necessary for the student or pharmacist to memorise the minimum dose, but it is obviously important to know the maximum. It is not necessary to memorise maximum doses in both metric and imperial systems, but during these years of transition to the metric system a good knowledge of equivalents is necessary. The following equivalents are given for this purpose, but it must be remembered that they are round figure approximations and must not be used as equivalents in weighing—

1 mg = $\frac{1}{60}$ grain	0.06 ml = 1 minim
5 mg = $\frac{1}{12}$ grain	1.0 ml = 15 minims
10 mg = $\frac{1}{6}$ grain	4.0 ml = 60 minims
30 mg = $\frac{1}{2}$ grain	30.0 ml = 1 fl oz
100 mg = 1½ grains	
300 mg = 5 grains	
500 mg = 8 grains	
1,000 mg = 15 grains	

**Doses Proportionate to Age**

There are two well known methods of calculating doses for children—

1 According to the formula invented by Young, viz.—

$$\frac{\text{Age in years}}{\text{Age in years} + 12} = \text{proportion of adult dose},$$

e.g. for a child of 6

$$\frac{6}{6 + 12} = \frac{1}{3} \text{ of adult dose}$$

2 According to the formula of W. J. Dilling, viz.—

$$\frac{\text{Age in years}}{20} = \text{proportion of adult dose},$$

e.g. for a child of 6

$$\frac{6}{20} = \frac{3}{10} \text{ of adult dose}$$

Dilling's method is probably better, in that mental calculation is usually quicker.

**Doses Proportionate to Body Weight**

The *Pharmacopoeia* includes several drugs the doses of which are based on body weight (see p. 583). For example, the dose of Chloramphenicol for a child is 25 to 50 mg per kg body weight, daily in divided doses.

If, therefore, a dose of 30 mg per kg body weight is prescribed for a child weighing 4 stone 12 lb the calculation would be as follows:

$$4 \text{ stone } 12 \text{ lb} = \frac{68}{22046} \text{ kg}$$

Therefore, the dose required is

$$\frac{68 \times 30}{22046} \text{ mg} = 925 \text{ mg}$$

**Doses Proportionate to Surface Area**

The methods based on age and body weight are not entirely satisfactory, and recently Catzel (1963) has devised a method based on the surface area of the body. As the dose to be given depends on such

factors as metabolic rate, lean body mass, and extracellular fluid volume, which are more closely related to surface area than to age or weight, the method would seem to be a reasonable one. It shows that children are sensitive to most drugs to about the same extent as adults. Exceptions are the lesser sensitivity of premature babies and young infants to phenobarbitone and the greater sensitivity to sulphonamides, opiates and certain antibiotics.

The method is not as simple as those based on age or weight, data which are easily obtained, but Catzel has produced tables giving ages, weights and a corresponding figure dependent on surface area. This figure is the percentage of the adult dose which should be used and is based on the following formula

$$\frac{\text{Surface area of child} \times 100}{\text{Surface area of adult}} = \text{percentage of adult dose}$$

Table A7.1 is adapted from *The Paediatric Prescriber* (2nd ed.) by P. Catzel and published in the *Drug and Therapeutic Bulletin*, Vol. 1, No. 18.

*Table A7.1*  
Percentage Method for Calculating Paediatric Doses

Weight lb kg	Average corresponding age	Percentage of adult dose
5½	2.5	One month premature
7	3.2	birth
10	4.5	2 months
14	6.5	4 months
22	10	12 months
25	11	18 months
33	15	3 years
40	18	5 years
50	23	7 years
66	30	10 years
80	36	11 years
88	40	12 years
100	45	14 years
120	54	16 years
145	65	20 years

**REFERENCES**

- HURST, A. F. (1935) Dosage above the Pharmacopoeial Maximum. *Pharm J.*, 135, 675, 703  
 CATZEL, P. (1963) *The Paediatric Prescriber* 2nd Ed  
 Blackwell Scientific Publications, Ltd.

## POSOLOGICAL TABLES

*Students may be expected to be familiar with the doses of drugs marked with an asterisk*

## IN PHARMACOPÆIAL ORDER

Name	Maximal Doses	Route etc	Name	Maximal Doses	Route etc
*Acetarsol	250 mg	Twice a day for ten days, in the treatment of intestinal amoebiasis.	Anise Oil	0·2 ml	
*Acetazolamide	500 mg	Initial dose	*Antazoline Hydrochloride	100 mg	
	250 mg	Subsequent doses every six hours	Antimony Potassium Tartrate	30 mg	Initial daily dose by intravenous injection, increasing by 30 mg every 48 hr to a maximum single dose of 120 mg until the total quantity administered is not less than 15 G
*Acetomenaphthone	10 mg	Daily for one week before delivery, in the prophylaxis of neonatal haemorrhage	Ascorbic Acid	8 mg	By subcutaneous or intramuscular injection.
	20 mg	Daily for one week, in the pre-operative treatment of obstructive jaundice	Atropine Methonitrate	75 mg	Daily prophylactic dose
	2 mg	In haemorrhagic disease of the new born, 1 mg for premature babies	*Apomorphine Hydrochloride	500 mg	Daily therapeutic dose
*Acetylsalicylic Acid	1 G		Atropine Sulphate	0·6 mg	As 6 ml of a 0·01 per cent aqueous solution half an hour before feeds, in the treatment of congenital hypertrophic pyloric stenosis
	8 G	Daily, in divided doses, in the treatment of acute rheumatism.	B C G Vaccine	2 mg	Orally and by subcutaneous or intramuscular injection
*Adrenaline Acid Tartrate	1 mg	By subcutaneous injection, as a single dose	*Barbitone Sodium	0·1 ml	Prophylactic, by intracutaneous injection as a single dose
Adrenaline Injection	0·5 ml	By subcutaneous injection, as a single dose	Belladonna Dry Extract	600 mg	
	0·05 ml	Per minute by subcutaneous injection, in the treatment of status asthmaticus and other allergic emergencies	*Belladonna Tincture	60 mg	
Aloes	300 mg		Prepared Belladonna Herb	2 ml	
Aluminium Hydroxide Gel	15 ml		*Bemegride	200 mg	
Dried Aluminium Hydroxide Gel	1 G			1 G (total)	50 mg at intervals of ten minutes, by intravenous injection in the treatment of barbiturate poisoning
*Aminophylline	300 mg	By slow intravenous injection	*Bendrofluozide	10 mg	Daily or on alternate days
	500 mg		Benzathine Penicillin	0·9 G	Prophylactic dose, by intramuscular injection, every two or three weeks
Ammonium Chloride	6 G	Daily, in divided doses, before the administration of Mersalyl Injection.	*Benzhexol Hydrochloride	2 mg	Initial dose, in divided doses, subsequent doses increasing gradually to 20 mg daily in accordance with the needs of the patient
Amodiaquine Hydrochloride	400 mg (base)	Suppressive dose, weekly or once every two weeks in the treatment of malaria	Benztropine Methanesulphonate	500 µg	Initial dose, daily, subsequent doses may be increased gradually to 6 mg daily in accordance with the needs of the patient
	600 mg (base)	Therapeutic dose, daily, for three days	Benzylpenicillin	500 mg	Every four hours.
*Amphetamine Sulphate	10 mg	Morning and midday		600 mg	By intramuscular injection, two to twelve times daily
*Amylobarbitone	200 mg		*Busulphan	4 mg	Daily
*Amylobarbitone Sodium	200 mg	Oral dose. When given by intravenous, intramuscular or subcutaneous injection the dose is determined by the physician in accordance with the needs of the patient.		2 mg	Daily maintenance dose
*Aneurine Hydrochloride	5 mg	Daily prophylactic dose.	*Butobarbitone	200 mg	
	100 mg	Daily therapeutic dose	Caffeine	500 mg	
	100 mg	By subcutaneous or intramuscular injection	Caffeine Hydrate	500 mg	

Name	Maximal Doses	Route etc	Name	Maximal Doses	Route etc
Calciferol	20 µg	Daily, in the prevention of rickets. Allowance being made for vitamin D obtained from other sources	*Chloroquine Sulphate	400 mg	Suppressive dose, weekly, in the treatment of malaria.
	1.25 mg	Daily, in the treatment of rickets and osteomalacia.		800 mg	Adult therapeutic dose, subsequent doses 400 mg daily
	5 mg	Daily, in the treatment of hypoparathyroidism.		300 mg (base)	Adult therapeutic dose, by intravenous or intramuscular injection
Calcium Amuno-salicylate	15 G	Daily, in divided doses	*Chlorothiazide	1.2 G	Initial dose, daily, in the treatment of discoid lupus erythematosus and rheumatoid arthritis.
Calcium Carbonate	5 G			200 mg	Maintenance dose, daily
Calcium Gluconate	5 G			200 mg	Daily dose, in the treatment of giardiasis
Calcium Lactate	5 G		*Chlorphenaramine Maleate	800 mg	Daily dose, in the treatment of hepatic amoebiasis
*Caramaphen Hydro-chloride	50 mg	Initial dose, daily in divided doses, subsequent doses increasing gradually to 600 mg daily in accordance with the needs of the patient.		2 G	Daily or on alternate days.
*Carbachol	4 mg			16 mg	Daily, in divided doses.
*Carbamazole	500 µg	By subcutaneous injection	*Chlorpromazine Hydrochloride	20 mg	By intramuscular injection, as a single dose
	60 mg	Controlling dose, daily, in divided doses.		500 mg	Daily, in divided doses, in psychiatric states
	20 mg	Maintenance dose, daily		50 mg	As an antiemetic
*Carbromal Compound Cardamom Tincture	1 G		*Chlorpropamide	500 mg	Orally, and by intramuscular injection
	5 ml			3 G	Daily
Cascara Dry Extract	250 mg				Daily, in divided doses.
Cascara Elixir	5 ml		*Chlortetracycline Hydrochloride		
Cascara Liquid Extract	5 ml				
Castor Oil	20 ml				
Chalk	5 G		*Codeine Phosphate Cod-liver Oil		
*Chloral Hydrate	2 G				
*Chlorambucil	10 mg	Daily			
*Chloramphenicol	4 mg	Maintenance dose	*Colchicine		
	3 G	Adult dose, daily, in divided doses. The dose for a child is calculated on body weight (see p. 583)			
*Chloreycyclizine Hydrochloride	200 mg	Daily			
Chloroform Spirit	2 ml		Colchicum Tincture	2 ml	
*Chloroquine Phosphate	500 mg	Suppressive dose, weekly, in the treatment of malaria	Coriander Oil	0.2 ml	
	1 G	Initial therapeutic dose, subsequent doses 500 mg daily	*Cortisone Acetate	400 mg	Daily, in divided doses, orally and by intramuscular injection
	300 mg (base)	Adult therapeutic dose, by intravenous or intramuscular injection.		50 mg	Daily, for replacement therapy after adrenalectomy, in Addison's disease, and in panhypopituitarism.
	1.5 G	Initial dose, daily, in the treatment of discoid lupus erythematosus and rheumatoid arthritis	*Cyanocobalamin	2 mg	By intramuscular injection, in divided doses in the first week, in the treatment of megaloblastic anaemia.
	250 mg	Maintenance dose, daily		250 µg	Subsequent doses, weekly, until the blood count is normal.
	250 mg	Daily dose, in the treatment of giardiasis.		250 µg	Maintenance dose, every three or four weeks. If the nervous system is involved the doses should be increased
	1 G	Daily dose, in the treatment of hepatic amoebiasis	*Cyclizine Hydrochloride	50 mg	
			*Cyclobarbitalone Calcium	400 mg	
			*Cycloserine	750 mg	Daily, in divided doses.

Name	Maximal Doses	Route etc	Name	Maximal Doses	Route etc
Dapsone	50 mg	Initial dose, twice weekly increasing by 50 to 100 mg every month to a maximum of 200 to 400 mg twice weekly	Dimercaprol	800 mg	By intramuscular injection in divided doses on the first day
Demethylchlortetracycline Hydrochloride	1.8 G	Daily, in divided doses		400 mg	In divided doses on the second and third day
*Deoxycortone Acetate	5 mg	By intramuscular injection daily		200 mg	In divided doses, on subsequent days
	400 mg	Total implantation dose	*Dimethisterone	40 mg	Daily, in divided doses
*Deoxycortone Tri-methylacetate	100 mg	By intramuscular injection once a month	*Diphenhydramine Hydrochloride	75 mg	By intramuscular or intra-venous injection, in single or divided doses
*Dexamethasone	10 mg	Daily, in divided doses		50 mg	By subcutaneous or intra-muscular injection
Dexamethasone Acetate		Daily, in divided doses	*Dipipanone Hydrochloride	50 mg	By injection three times a week or 1.5 G daily
Dexamphetamine Sulphate	10 mg	Morning and midday	Ditophal	5 G	
*Dextromethorphan Hydrobromide	30 mg		Edrophonium Chloride	2 mg	By intravenous injection followed if no response occurs within thirty seconds, by 8 mg
*Diamorphine Hydrochloride	10 mg	Orally and by subcutaneous or intramuscular injection	Emetine and Bismuth Iodide	200 mg	Daily
*Dichlorphenamide	50 mg	One to four times daily	*Emetine Hydrochloride	60 mg	By subcutaneous or intra-muscular injection daily
*Dienoestrol	5 mg	Daily, in the treatment of menopausal symptoms	*Ephedrine Hydrochloride	60 mg	
	15 mg	Three daily for three days, followed by 15 mg daily for six days, for the suppression of lactation	*Ergometrine Maleate	1 mg	Orally and by intramuscular injection
	30 mg	Daily, in the treatment of carcinoma of the prostate and mammary carcinoma	*Ergotamine Tartrate	500 µg	By intravenous injection
Diethazine Hydrochloride	150 mg	Initial dose, daily in divided doses, subsequent doses increasing gradually to 1.5 G daily in accordance with the needs of the patient		2 mg	As a single dose
				500 µg	By subcutaneous or intra-muscular injection
Diethylcarbamazine Citrate	500 mg	Daily	*Erythromycin Estolate	2 G	Daily in divided doses
*Prepared Digitalis	1.5 G	Initial dose, in divided doses, for rapid digitalisation		2 G	Daily in divided doses for not more than ten days
	200 mg	Maintenance dose, daily	*Ethynodiol Diacetate	(base) 50 µg	Daily in the treatment of menopausal symptoms.
Digoxin	1.5 mg	Initial dose, in divided doses, for rapid digitalisation.		100 µg	Three daily for three days followed by 100 µg daily for six days, for the suppression of lactation
	200 µg	Maintenance dose, daily		2 mg	Daily in the treatment of carcinoma of the prostate and mammary carcinoma
	1.5 mg	Initial dose, in single or divided doses for rapid digitalisation	*Ethisterone	100 mg	Daily
	250 µg	Maintenance dose one to three times daily	*Ethopropazine Hydrochloride	50 mg	Initial dose, increasing gradually to 500 mg daily in accordance with the needs of the patient
	1 mg	By intramuscular or slow intravenous injection for rapid digitalisation in patients who have not been given cardiac glycosides within the preceding two weeks	Ethotoxin	1 G	Daily, increasing to 3 G in accordance with the needs of the patient
Diiodohydroxy-quinoline	2 G	Daily for twenty days	Ferric Ammonium Citrate	3 G	
Diloxanide Furoate	1.5 G	Daily for ten days or more	*Ferrous Gluconate	300 mg	Prophylactic daily dose
*Dimenhydrinate	100 mg			2.4 G	Therapeutic daily dose, in divided doses
	50 mg	By intramuscular injection	*Ferrous Sulphate	300 mg	Prophylactic daily dose
				1.8 G	Therapeutic daily dose, in divided doses
			*Dried Ferrous Sulfate	200 mg	Prophylactic daily dose
				1.2 G	Therapeutic daily dose, in divided doses

Name	Maximal Doses	Route etc	Name	Maximal Doses	Route etc
*Fludrocortisone Acetate	2 mg 200 µg	In the treatment of acute adrenocortical insufficiency Maintenance dose daily	*Hydroxocobalamin	2 mg	By intramuscular injection in divided doses in the first week in the treatment of megaloblastic anaemia.
Fluoxymesterone Folic Acid	20 mg 20 mg	Daily Daily		250 µg	Subsequent doses weekly until the blood count is normal
Compound Gentian Tincture	5 ml			250 µg	Maintenance dose, every three or four weeks
Concentrated Compound Gentian Infusion	5 ml		Hydroxychloroquine Sulphate	400 mg 800 mg	Suppressive dose weekly, in the treatment of malaria. Initial therapeutic dose subsequent doses 400 mg daily
Compound Gentian Infusion	40 ml			12 G	Initial dose daily in the treatment of discoid lupus erythematosus and rheumatoid arthritis
Strong Ginger Tincture	0.5 ml			200 mg 200 mg	Maintenance dose daily Daily in the treatment of giardiasis
Ginger	1 G		*Hyoscine Hydrobromide	600 µg	Orally and by subcutaneous injection
Ginger Syrup	5 ml		Hyoscyamus Dry Extract	60 mg	
Weak Ginger Tincture	3 ml		Hyoscyamus Liquid Extract	0.5 ml	
*Glutethimide	500 mg		*Hyoscyamus Tincture	5 ml	
*Glyceryl Trinitrate	1 mg				
*Gnosefolvin (fine particle)	1 G	Daily in divided doses			
*Guanethidine Sulfate	10 mg	Initial dose daily subsequent doses increasing at weekly intervals to a maximum of 300 mg daily in accordance with the needs of the patient	*Imipramine Hydrochloride	150 mg	Daily in divided doses
Halibut liver Oil	0.5 ml	Daily	Indigo Carmine	80 mg	By intravenous injection
*Hexamethonium Tartrate	15 mg	Initial dose by subcutaneous injection every six hours subsequent doses increasing gradually to a maximum of 400 mg daily in accordance with the needs of the patient	Aqueous Iodine Solution	1 ml	In the pre-operative treatment of thyrotoxicosis
*Hexylresorcinol	1 G	Single dose for adults in the treatment of ascaris infestation	Iopanoic Acid	6 G	As a single dose ten to fifteen hours before radiographic examination
	100 mg	For each year of age up to 10 years as a single dose	Ipecacuanha Liquid Extract	0.1 ml	
*Histamine Acid Phosphate	1 mg 5 mg	By subcutaneous injection. After the administration of an antihistamine.	*Ipecacuanha Tincture	1 ml 20 ml	As an emetic
*Hydralazine Hydrochloride	50 mg	Initial dose daily in divided doses, subsequent doses may be increased gradually to a maximum of 200 mg daily in accordance with the needs of the patient	Prepared Ipecacuanha	125 mg	
Dilute Hydrochloric Acid	10 ml		*Ipecacuanha and Opium Powder	600 mg	
*Hydrochlorothiazide	100 mg	Daily or on alternate days.	*Isoniazid	600 mg	Daily in divided doses
*Hydrocortisone Acetate	50 mg	By intra-articular injection	*Isoprenaline Sulphate	20 mg	Sublingually or by inhalation of a solution by means of an atomiser
Hydrocortisone Sodium Succinate	= 50 mg	By intravenous injection repeated if necessary in accordance with the needs of the patient.	Light Kaolin	75 G 2 mg	By intravenous injection
Hydroflumethiazide	100 mg	Daily or on alternate days.	*Levorphanol Tartrate	4.5 mg 4 mg	By subcutaneous or intra muscular injection
			*Lothyronine Sodium	1.5 mg 100 µg	By intravenous injection Daily in accordance with the age condition and response of the patient.
			Liquorice Liquid Extract	5 ml	
			Lucanthone Hydrochloride	1 G	Twice daily for three days

Name	Maximal Doses	Route etc	Name	Maximal Doses	Route etc
Light and Heavy Magnesium Carbonate	500 mg 5 G	As an antacid As a laxative	Methylene Blue		Dose calculated on body weight (see p 583)
Light Magnesium Oxide	500 mg 5 G	As an antacid As a laxative	*Methylergometrine Maleate	500 µg 200 µg	By subcutaneous intramuscular or intravenous injection
Magnesium Hydroxide Mixture	10 ml 50 ml	As an antacid As a laxative	Methylprednisolone	80 mg 50 mg 20 mg	Daily, in divided doses Daily for a man Daily for a woman
Magnesium Sulphate	15 G		*Methyltestosterone	100 mg	Daily, in the treatment of mammary carcinoma
Magnesium Trisilicate	2 G		*Methylthiouracil	600 mg 200 mg	Daily controlling dose Daily maintenance dose
Male Fern Extract	6 ml	After fasting for twenty four hours	Methyprylon	400 mg 400 mg	As a hypnotic As a sedative, daily in divided doses
*Mecamylamine Hydrochloride	2.5 mg	Initial dose twice a day subsequent doses may be increased gradually to a maximum of 60 mg daily in accordance with the needs of the patient.	*Morphine Hydrochloride	20 mg	
*Meclozine Hydrochloride	50 mg	Daily	Morphine Hydrochloride Solution	2 ml	
*Melarsoprol		Dose calculated on body weight (see p 583)	*Morphine Sulphate	20 mg	Orally and by subcutaneous or intramuscular injection
*Menaphthone	10 mg	By intramuscular injection daily in accordance with the prothrombin activity of the blood	*Mustine Hydrochloride		Dose calculated on body weight (see p 583)
*Menaphthone Sodium B sulphate	2 mg up to 50 mg 1 mg	By subcutaneous or intramuscular injection daily in accordance with the prothrombin activity of the blood In an emergency every four hours By subcutaneous injection as a single dose in the treatment of the new born	*Nalorphine Hydrobromide	10 mg	Initial dose by intravenous injection, repeated in accordance with the needs of the patient to a total dose not exceeding 40 mg
Mepacrine Hydrochloride	100 mg 500 mg 1 G	Suppressive daily dose in the treatment of malaria Therapeutic daily dose in divided doses In divided doses in the treatment of tapeworm infestation.	*Nandrolone Phenylpropionate	50 mg	By intramuscular injection weekly
*Mephentermine Sulphate	= 80 mg (base)	By intramuscular or slow intravenous injection	Neomycin Sulphate	8 mega units	Daily in divided doses, as an intestinal antiseptic
*Meprobamate	1.2 G	Daily in divided doses	*Neostigmine Bromide	30 mg	The frequency of the dose being determined by the needs of the patient.
*Mepyramine Maleate	200 mg		*Neostigmine Methylsulphate	2 mg	By subcutaneous or intramuscular injection the frequency of the dose being determined in accordance with the needs of the patient
*Mercaptopurine Mersalyl Injection	200 mg 2 ml	Daily By intramuscular injection on alternate days	*Nicotinamide	30 mg 250 mg 250 mg	Daily prophylactic dose Daily therapeutic dose By intravenous injection daily
*Methadone Hydrochloride	10 mg	Orally and by subcutaneous injection	*Nicotinic Acid	30 mg 250 mg	Daily prophylactic dose Daily therapeutic dose
Methandienone	10 mg	Daily	Nikethamide	2 G	By intravenous injection
*Methocillin Sodium	1 G	By intramuscular injection on every four hours	*Nitrofurantoin	150 mg	Four times daily
*Methion	100 mg	Daily increasing to 600 mg in accordance with the needs of the patient.	Noradrenaline Acid Tartrate	20 µg per minute	By intravenous infusion according to the blood pressure of the patient.
Methylamphetamine Hydrochloride	10 mg 30 mg	By intramuscular or intravenous injection.	Norethandrolone	50 mg	Daily
Methylcellulose 450	4 G	Daily, in divided doses	Norethisterone	30 mg	Daily in divided doses
			*Noscapine	30 mg	
			Novobiocin Calcium and Novobiocin Sodium	= 2 G novobiocin	
			Nux Vomica Liquid Extract	0.2 ml	Daily in divided doses
			*Nux Vomica Tincture	2 ml	

Name	Maximal Doses	Route etc	Name	Maximal Doses	Route etc
*Oestradiol Benzoate	5 mg	By intramuscular injection, daily	Phenelzine Sulphate	= 45 mg phenel zine	Daily
*Opium Tincture	2 ml		*Phenindamine Tartrate	50 mg	
*Camphorated Opium Tincture	10 ml		*Phenindione		
Powdered Opium	200 mg		*Phenobarbitone	300 mg	Initial dose
Orange Tincture	2 ml		*Phenobarbitone Sodium	100 mg	Subsequent doses daily, in accordance with the prothrombin activity of the blood
Orange Syrup	5 ml				
*Oxytetracycline Di-hydrate	3 G	Daily, in divided doses			
*Oxytetracycline Hydrochloride	2 G	By intravenous infusion daily, in a concentration not exceeding 0.1 per cent w/v	*Phenolphthalein	120 mg	
Pancreatin	3 G		Phenolsulphonaphthalein	120 mg	
*Paracetamol	1 G		*Phenoxybenzamine Hydrochloride	200 mg	By intravenous or intramuscular injection as a single dose
Liquid Paraffin	30 ml	Orally and by intramuscular injection.	*Phenoxyethylpenicillin	300 mg	
*Paraldehyde	8 ml	By rectal injection as a basal anaesthetic.	Phenoxyethylpenicillin Calcium	6 mg	By intravenous injection
*Paramethadione	900 mg	Daily in divided doses for an adult, increasing to 1.8 G in accordance with the needs of the patient.	Phenoxyethylpenicillin Potassium	= 250 mg	Initial dose, subsequent doses increasing in accordance with the needs of the patient.
	300 mg	Daily, in divided doses for a child, increasing to 900 mg in accordance with the needs of the patient.	Phentolamine Hydrochloride	250 mg	Every four hours
*Pempidine Tartrate	2.5 mg	Initial dose, every six hours subsequent doses may be increased gradually to a maximum of 80 mg daily in accordance with the needs of the patient.	*Phentolamine Methanesulphonate	400 mg	Every four hours
*Pentaerithritol tetra-nitrate	30 mg		*Phenylbutazone	5 mg	Daily, in divided doses
Pentamidine Isethionate	300 mg	By intramuscular injection every three to six months in the prophylactic treatment of trypanosomiasis	*Phenylephrine Hydrochloride	500 µg	By subcutaneous or intramuscular injection
	300 mg	By intramuscular injection daily for seven to fifteen days in the early treatment of trypanosomiasis and leishmaniasis	*Phenytoin Sodium	50 mg	By intravenous injection
*Pentobarbitone Sodium	200 mg		*Pholcodine Dilute Phosphoric Acid	15 mg	Daily, increasing to 200 mg in accordance with the needs of the patient.
*Pentolinium Tartrate	1 mg	Initial dose by subcutaneous injection, subsequent doses increasing gradually in accordance with the needs of the patient.	*Phthalylsulphathiazole	5 mg	
Peppermint Oil Concentrated Peppermint Water	0.2 ml 1 ml		*Phytomenadione	10 G	Daily in divided doses
*Perphenazine	24 mg	Daily, in divided doses in psychiatric states.		20 mg	As an antidote to anticoagulants except heparin
	8 mg	As an antiemetic.		150 mg	By intravenous injection repeated in a few hours.
*Pethidine Hydrochloride	100 mg	Orally and by subcutaneous or intramuscular injection.		10 mg	By intravenous injection in the treatment of haemorrhagic disease of the newborn
	50 mg	By intravenous injection.		2 mg	Prophylactic for the mother by mouth.
Phanquone	300 mg	Daily, in divided doses for ten days.	*Picrotoxin	6 mg	Initial dose by intravenous injection in the treatment of barbiturate poisoning.
Phenacetin	600 mg		*Piperazine Adipate	2 G	Daily, in divided doses, for an adult, in the treatment of threadworm infestation
			*Piperazine Citrate		Single dose as an ascarcide
			*Piperazine Phosphate	4.5 G	Children's doses given according to bodyweight (see p. 583)

Name	Maximal Doses	Route etc	Name	Maximal Doses	Route etc
Polymyxin B Sulphate	2 mega units 500 000 units	Daily, in divided doses By intramuscular injection every eight hours	Pyndostigmine Bromide	240 mg	The frequency of the dose being determined in accordance with the needs of the patient
Potassium Bromide	6 G	Daily, in divided doses		5 mg	By intramuscular or subcutaneous injection, the frequency of the dose being determined in accordance with the needs of the patient
Potassium Citrate	2 G				
Potassium Iodide	500 mg 50 mg	As an expectorant In the pre-operative treatment of thyrotoxicosis			
*Potassium Perchlorate	800 mg	Controlling dose, daily, in divided doses	*Pyridoxine Hydrochloride	150 mg	Daily, in divided doses, in pyridoxine-dependent anaemia of adults
*Prednisolone	400 mg	Maintenance dose, daily			Children's doses according to body-weight, in pyridoxine-dependent convulsions in infancy (see p. 583)
Prednisolone Acetate	100 mg	Daily, in divided doses	*Pyrimethamine	50 mg	Weekly, as a suppressive of malaria
*Prednisolone Tri-methylacetate	100 mg	Daily, in divided doses			
*Prednisone	20 mg	By intra-articular injection	*Quinalbarbitone Sodium	200 mg	
Prednisone Acetate	100 mg		*Quinidine Sulphate	200 mg	Three or four times a day, in the prophylaxis of cardiac arrhythmias
*Primaquine Phosphate	= 60 mg (base) = 15 mg (base)	Once a week in association with a schizonticide for the prophylaxis of malaria Daily for fourteen days for the radical cure of <i>P. vivax</i> malaria		400 mg	Every two to four hours to a total of 3 G daily, in the treatment of atrial fibrillation
*Primidone	0.5 G	Daily, increasing to 2 G in accordance with the needs of the patient	Quinine Bisulphate	600 mg	
Probenecid	2 G	Daily	Quinine Dihydrochloride	600 mg	Orally and by intravenous injection
*Procainamide Hydrochloride	1.5 G	Also by slow intravenous injection of a 2.5 per cent w/v solution of an amount determined by the effects produced	Quinine Hydrochloride	600 mg	
*Procaine Penicillin	900 mg	By intramuscular injection, daily	*Quinine Sulphate	600 mg	
*Prochlorperazine Maleate	100 mg	Daily, in divided doses, in psychiatric states	*Reserpine	5 mg	Daily, in divided doses, in psychiatric states
	30 mg	As an antiemetic		500 µg	Daily, in the treatment of hypertension
*Prochlorperazine Methanesulphonate	25 mg	By intramuscular injection, two or three times a day, in psychiatric states	Rhubarb Compound Rhubarb Tincture	1 G 5 ml	
	12.5 mg	By intramuscular injection as an antiemetic	*Riboflavin	4 mg 10 mg	Daily prophylactic dose Daily therapeutic dose
*Procyclidine Hydrochloride	7.5 mg	Initial dose, daily, in divided doses, subsequent doses increasing gradually to 30 mg daily, in divided doses, in accordance with the needs of the patient	Santonin	200 mg	Daily, for three days
			Senna Fruit	2 G	
*Progesterone	60 mg	By intramuscular injection, daily	Senna Leaf	2 G	
*Proguanil Hydrochloride	300 mg	Daily as a suppressive of malaria	Smallpox Vaccine	about 0.02 ml	Prophylactic by scarification or pressure inoculation
*Promazine Hydrochloride	800 mg	Orally and by intramuscular or intravenous injection, daily, in divided doses	Sodium Amino-salicylate	15 G	Daily in divided doses
*Promethazine Hydrochloride	50 mg	Daily, orally and by intramuscular injection	Sodium Anoxynaphthonate	100 mg	By intravenous injection
			Sodium Antimony gluconate		Dose calculated on body weight (see p. 583)
*Promethazine Thecolate	50 mg	Daily	*Sodium Aurothiomalate	10 mg	By intramuscular injection, increasing gradually to 100 mg weekly. The total dose in one course of treatment should not usually exceed 1 G
*Propantheline Bromide	30 mg				
Propylthiouracil	600 mg 200 mg	Controlling dose, daily Maintenance dose, daily			

Name	Maximal Doses	Route etc	Name	Maximal Doses	Route etc
Sodium Bicarbonate	5 G	As an antacid	*Sulphafurazole	3 G	Initial dose in the treatment of systemic infections, subsequent doses up to 6 G daily, in divided doses.
Sodium Bromide	6 G	Daily, in divided doses		2 G	Initial dose in the treatment of infections of the urinary tract, subsequent doses up to 4 G daily, in divided doses.
*Sodium Calcium editate	4 G	Daily in divided doses as required. Intravenous dose calculated on body weight (see p 583)			
Sodium Citrate	4 G				
Sodium Iodide	500 mg 50 mg	As an expectorant. In the pre-operative treatment of thyrotoxicosis	*Sulphamethizole Sulphamethoxy pyridazine	200 mg 1 G	Every four to six hours Initial dose, subsequent doses 500 mg daily
Sodium Salicylate	10 G	Daily, in divided doses, in the treatment of acute rheumatism.	*Sulphasomidine	3 G	Initial dose in the treatment of systemic infections, subsequent doses up to 6 G daily, in divided doses.
Sodium Stibogluconate	2 G	By intramuscular or intravenous injection, daily for ten to thirty days		4 G	Daily, in divided doses, in the treatment of infections of the urinary tract.
Sodium Sulphate	15 G				Dose calculated on body weight (see p 583)
*Solapsone	3 G 125 mg	Daily By intramuscular injection twice a week, increasing to a maximum dose of 1.5 G Given in a 50 per cent w/v solution.	Sulphobromophthalain Sodium Suranum	500 mg	Initial dose by intravenous injection, subsequent doses 2 G weekly for five weeks.
Subophen	300 mg	By intramuscular or intravenous injection, daily, or at longer intervals to a total dose of 4.5 G	*Testosterone *Testosterone Phenyl propionate	600 mg 25 mg	Total implantation dose. By intramuscular injection, once or twice weekly
*Sulboestrol	1 mg 5 mg 20 mg	Daily, in the treatment of menopausal symptoms. Thrice daily for three days, followed by 5 mg daily for six days, for the suppression of lactation. Daily, in the treatment of carcinoma of the prostate and in mammary carcinoma.	*Testosterone Propionate *Tetrachlorethylene *Tetracycline Hydrochloride	25 mg 3 ml 3 G 2 G	By intramuscular injection, once or twice weekly As a single dose Daily, in divided doses. By intravenous infusion, daily, in a concentration not exceeding 0.5 per cent w/v
Stramonium Liquid Extract	0.2 ml		Thiambutosine	500 mg	Daily, increasing every fourteen days by 500 mg to a maximum dose of 3 G daily
*Stramonium Tincture	2 ml		*Thiopentone Sodium	500 mg 2 G	By intravenous injection. Maximum dose by rectal injection calculated on body weight (see p 583)
*Streptomycin Sulphate	= 500 mg (base) = 1 G (base)	Every eight hours, as an intestinal antiseptic By intramuscular injection, daily or at longer intervals.	Thiordiazine Hydrochloride *Thyroid	600 mg 250 mg	Daily, in divided doses Daily
*Succinylsulphathiazole	20 G	Daily, in divided doses.	*Thyroxine Sodium *Tolazolol Hydrochloride *Tolbutamide Tolu Syrup	300 µg 75 mg 1.5 G 10 ml	Daily Daily Daily Daily
Sulphadiazine	3 G	Initial dose subsequent doses up to 4 G daily, in divided doses.	*Trifluoperazine Hydrochloride	30 mg	Daily, in divided doses in psychiatric states.
*Sulphadimidine	3 G 2 G	Initial dose in the treatment of systemic infections, subsequent doses, up to 6 G daily, in divided doses. Initial dose in the treatment of infections of the urinary tract, subsequent doses up to 4 G daily, in divided doses	Triptenamine Hydrochloride *Tozidone	6 mg 100 mg 900 mg	As an antiemetic daily Adult dose, daily, in divided doses, increasing to 1.8 G in accordance with the needs of the patient.
*Sulphadimidine Sodium	2 G	By intramuscular or intravenous injection.		300 mg	Child's dose, daily, in divided doses, increasing to 900 mg in accordance with the needs of the patient.

Name	Maximal Doses	Route etc		IN ASCENDING ORDER OF DOSE
Tryparsamide	3 G	By subcutaneous, intramuscular or intravenous injection at intervals of five to seven days to a total of 24 G		MAXIMUM OFFICIAL DOSES
Urethane	3 G	Daily		Where a drug has more than one dose these are given together, the first being that given first in the <i>Pharmacopæia</i> even when it is not the smallest.
Viomycin Hydrochloride	2 mega units	By slow intravenous injection daily, in single or divided doses in a concentration not exceeding 0.5 per cent w/v	20 µg	
Viomycin Sulphate	1 mega unit	By intramuscular injection, daily or at longer intervals		Calciferol
*Warfarin Sodium	50 mg	Initial dose, subsequent doses 10 mg daily, in accordance with the prothrombin activity of the blood	1.25 mg	Not more than 20 µg daily in the prevention of rickets, allowance being made for vitamin D obtained from other sources
			5 mg	Daily in the treatment of rickets and osteomalacia
			Noradrenaline Acid Tartrate	Daily in the treatment of hypoparathyroidism
				By intravenous infusion, per minute, according to the blood pressure of the patient.

### DOSES CALCULATED ON BODY WEIGHT

Name	Maximum dose per kg body weight	Route etc		
Chloramphenicol	50 mg	Daily, in divided doses for a child	50 µg	*Ethinylestradiol
Melarsoprol	3.6 mg	Adult dose by intravenous injection daily, for three days in the treatment of advanced trypanosomiasis	100 µg	Daily, in the treatment of menopausal symptoms.
	1.8 mg	Dose for a child, daily for three days	2 mg	Thrice daily for three days followed by 100 µg daily for six days, for the suppression of lactation
Methylene Blue	4 mg	Repeat after ten days		Daily, in the treatment of carcinoma of the prostate and mammary carcinoma.
Mustine Hydrochloride	400 µg	By intravenous injection.		
Piperazine Adipate Piperazine Citrate	= 40 mg hydrate	As a single dose by intravenous injection or in divided doses over four days	100 µg	Daily, in accordance with the age, condition and response of the patient
Piperazine Phosphate	= 120 mg hydrate	Daily, in divided doses for a child, in the treatment of threadworm infestation.	300 µg	
Pyridoxine Hydrochloride	4 mg	Single dose for a child as an ascarcide, up to a maximum of 4 G	500 µg or 0.5 ml	
Sodium Antimonyl gluconate	3.3 mg	Daily, for short periods, in pyridoxine-dependent convulsions in infancy	Benztropine Methanesulphonate	Initial dose daily, subsequent doses may be increased gradually to 6 mg daily in accordance with the needs of the patient.
Sodium Calcium edetate	= 40 mg anhydrous salt	By intravenous injection daily, for six to twelve days	Halbut Liver Oil *Methylergometrine Maleate	Daily
Sulphobromophthalein Sodium	5 mg	By intravenous injection.	200 µg	
Thiopentone Sodium	40 mg	By rectal injection. Maximum dose 2 G	600 µg	By subcutaneous, intramuscular, or intravenous injection
			*Hyoscine Hydrobromide	Orally and by subcutaneous injection
			1 mg	As a single dose by subcutaneous injection
			*Adrenaline Acid Tartrate	
			*Colchicine	Initial dose
			500 µg	Subsequent doses every two hours until relief of pain is obtained or vomiting or diarrhoea results.
			*Ergometrine Maleate	Orally and by intramuscular injection
			500 µg	By intravenous injection.

1 mg (continued)				
*Glyceryl Trinitrate	Tablets to be dissolved slowly in the mouth.	*Hydroxocobalamin	By intramuscular injection, in divided doses, in the first week, in the treatment of megaloblastic anaemia.	
*Histamine Acid Phosphate	By subcutaneous injection.	250 µg	Subsequent doses weekly until the blood count is normal.	
5 mg	After the administration of an antihistamine.	250 µg	Maintenance dose every three or four weeks.	
Pentolinium Tartrate	Initial dose by subcutaneous injection, subsequent doses, increasing gradually in accordance with the needs of the patient.	*Levallophan Tartrate	By intravenous injection.	
		*Menaphthone Sodium Bisulphite	By subcutaneous or intramuscular injection, daily, in accordance with the prothrombin activity of the blood	
Sulboestrol	Daily in the treatment of menopausal symptoms.	Up to 50 mg	In an emergency, every four hours, in accordance with the prothrombin activity of the blood	
5 mg	Three daily for three days, followed by 5 mg daily for six days, for the suppression of lactation.	1 mg	As a single dose, by subcutaneous injection in the treatment of the new born.	
20 mg	Daily in the treatment of carcinoma of the prostate and mammary carcinoma.	*Neostigmine Methyl sulphate	By subcutaneous or intramuscular injection, the frequency of the dose being determined in accordance with the needs of the patient.	
1.5 mg		2.5 mg		
*Digoxin	Initial dose, in single or divided doses for rapid digitalisation.	*Mecamylamine Hydrochloride	Initial dose twice a day. Subsequent doses may be increased gradually to a maximum of 60 mg daily in accordance with the needs of the patient.	
250 µg	Maintenance dose, one to three times daily.	*Pempidine Tartrate	Initial dose every six hours. Subsequent doses may be increased gradually to a maximum of 30 mg daily in accordance with the needs of the patient.	
1 mg	By intramuscular or slow intravenous injection, for rapid digitalisation in patients who have not been given cardiac glycosides within the preceding two weeks.	4.0 mg		
2 mg	Only and by subcutaneous or intramuscular injection.	*Busulphan	Daily Maintenance dose, daily	
*Atropine Sulphate		*Carbachol	By subcutaneous injection.	
*Benzhexol Hydrochloride	Initial dose in divided doses subsequent doses increasing gradually to 20 mg daily in accordance with the needs of the patient.	500 µg	Daily prophylactic dose.	
		*Riboflavin	Daily therapeutic dose.	
*Cyanocobalamin	By intramuscular injection, in divided doses, in the first week, in the treatment of megaloblastic anaemia. Subsequent doses, weekly, until the blood count is normal.	4.5 mg		
250 µg	Maintenance dose, every three or four weeks. If the nervous system is involved the doses should be increased.	*Levorphanol Tartrate	By subcutaneous or intramuscular injection.	
250 µg	By intravenous injection. Given within thirty seconds of the 2 mg dose if no response occurs.	4 mg	By intravenous injection.	
Edrophonium Chloride 8 mg	As a single dose.	1.5 mg		
	By subcutaneous or intramuscular injection.	5.0 mg		
*Ergotamine Tartrate 500 µg	In the treatment of acute adenocortical insufficiency	*Aneurine Hydrochloride	Daily prophylactic dose.	
*Fludrocortisone Acetate 200 µg	Daily maintenance dose	100 mg	Daily therapeutic dose.	
		100 mg	By subcutaneous or intramuscular injection.	
		*Deoxycortone Acetate	By intramuscular injection, daily.	
		400 mg	Total implantation dose.	
		*Dienoestrol	Daily in the treatment of menopausal symptoms.	

15 mg	Thrice daily for three days, followed by 15 mg daily for six days, for the suppression of lactation	*Methadone Hydrochloride 10 mg	By subcutaneous injection. Daily
30 mg	Daily in the treatment of carcinoma of the prostate and mammary carcinoma	Methandienone Methylamphetamine Hydrochloride 30 mg	By intramuscular or intravenous injection.
*Oestradiol Benzoate	By intramuscular injection, daily	*Nalorphine Hydrobromide	Initial dose by intravenous injection, repeated in accordance with the needs of the patient to a total dose not exceeding 40 mg
*Phenylephrine Hydrochloride	By subcutaneous or intramuscular injection	*Phentolamine Methanesulphonate	By intravenous injection
500 µg	By intravenous injection	*Sodium Aurothiomalate	By intramuscular injection, increasing gradually to 100 mg weekly. The total dose in one course of treatment should not usually exceed 1 G
*Reserpine	Daily, in divided doses in psychiatric states	15 mg	Initial dose, by subcutaneous injection, every six hours, subsequent doses increasing gradually to a maximum of 400 mg daily in accordance with the needs of the patient
500 µg	Daily, in the treatment of hypertension	*Hexamethonium Tartrate	Initial dose, by subcutaneous injection, every six hours, subsequent doses increasing gradually to a maximum of 400 mg daily in accordance with the needs of the patient
60 mg	Phenolsulphonphthalein	16 mg	*Pholcodine
*PicROTOxin	By intravenous injection	*Chlorpheniramine Maleate 20 mg	Daily in divided doses
	Initial dose, by intravenous injection, in the treatment of barbiturate poisoning	20 mg	By intramuscular injection as a single dose
7.5 mg	*Procyclidine Hydrochloride	Fluoxymesterone Folic Acid *Isoprenaline Sulphate	Daily
	Initial dose, daily, in divided doses, subsequent doses gradually increasing to 30 mg daily, in divided doses, in accordance with the needs of the patient	*Morphine Hydrochloride *Morphine Sulphate	Daily
8.0 mg	*Apomorphine Hydrochloride	*Phenoxybenzamine Hydrochloride	Soblinally or by inhalation of a solution by means of an atomiser
10 mg	*Acetomenaphthone	*Phytomenadione	As an anticoagulant except heparin
	Daily, for one week before delivery, in the prophylaxis of neonatal haemorrhage	150 mg	By intravenous injection, repeated in a few hours
20 mg	Daily, for one week, in the pre-operative treatment of obstructive jaundice	10 mg	By intravenous injection in the treatment of haemorrhagic disease of the new born
2 mg	In haemorrhagic disease of the new born	2 mg	Prophylactic for the mother, by mouth
1 mg	For premature babies	*Prednisolone Trimethyl acetate	By intra articular injection
*Amphetamine Sulphate	Morning and midday	*Perphenazine 8 mg	Daily, in divided doses, in psychiatric states
*Bendrofluazide	Daily or on alternate days	24 mg	As an antiepileptic
*Chlorambucil	Daily	*Prochlorperazine Methanesulphonate	By intramuscular injection two or three times a day in psychiatric states
4 mg	Maintenance dose		
Cod liver Oil	Not exceeding 10 mg daily in the prevention of rickets, allowance being made for vitamin D obtained from other sources		
*Dexamethasone	Daily in divided doses		
Dexamethasone Acetate	Daily in divided doses		
Dexamphetamine Sulphate	Morning and midday		
*Diamorphine Hydrochloride			
10 mg	By subcutaneous or intramuscular injection		
*Guanethidine Sulphate	Initial daily dose, subsequent doses, increasing at weekly intervals to a maximum of 300 mg daily in accordance with the needs of the patient		
*Menaphthone	By intramuscular injection, daily, in accordance with the prothrombin activity of the blood		

25 mg (continued)			*Hydrocortisone Acetate	By intra articular injection.
	12.5 mg	By intramuscular injection as an antiemetic.	Hydrocortisone Sodium	By intravenous injection, repeated, if necessary, in accordance with the needs of the patient.
*Testosterone Phenylpropionate		By intramuscular injection, once or twice weekly	Succinate (equivalent of 50 mg hydrocortisone)	
*Testosterone Propionate		By intramuscular injection, once or twice weekly	Meclozine Hydrochloride	Daily
30 mg			*Methyltestosterone	Daily for a man. Daily for a woman.
Antimony Potassium Tartrate		Initial daily dose by intra venous injection, increasing by 30 mg every forty-eight hours to a maximum single dose of 120 mg until the total quantity administered is not less than 1.5 G	20 mg 100 mg	Daily, in the treatment of mammary carcinoma.
Antimony Sodium Tartrate			*Nandrolone Phenylpropionate	By intramuscular injection, weekly
*Dextromethorphan Hydrobromide			Norethandrolone	Daily
*Neostigmine Bromide		The frequency of the dose is determined in accordance with the needs of the patient.	*Phenindamine Tartrate	
*Nicotinic Acid 250 mg		Daily prophylactic dose.	*Phenytoin Sodium	Daily, increasing to 200 mg in accordance with the needs of the patient.
*Nicouanamide 250 mg		Daily therapeutic dose.	*Promethazine Hydrochloride	Orally and by intramuscular injection, daily
250 mg		Daily prophylactic dose.	*Promethazine Thecolate	Daily
Norethisterone		Daily therapeutic dose.	*Pyrimethamine	Weekly, as a suppressive of malaria.
*Noscapine		By intravenous injection, daily	*Warfarin Sodium	Initial dose, subsequent doses 3 to 10 mg daily, in accordance with the prothrombin activity of the blood.
*Pentaerythritol Tetra nitrate		Daily, in divided doses.	60 mg	
*Propantheline Bromide			Belladonna Dry Extract	
*Trifluoperazine Hydrochloride	6 mg	Daily in divided doses, in psychiatric states.	*Carbamazole	Controlling dose, daily, in divided doses.
40 mg		Daily, as an antiemetic	20 mg	Maintenance dose, daily
*Dimethisterone		Daily, in divided doses.	*Codeine Phosphate	
45 mg			*Emetine Hydrochloride	By subcutaneous or intramuscular injection, daily
Phenelzine Sulphate		The equivalent of 45 mg of phenelzine, daily	*Ephedrine Hydrochloride	
50 mg			Hyoscyamus Dry Extract	
*Bemegride		By intravenous injection at intervals of ten minutes up to a total dose not exceeding 1 G, in the treatment of barbiturate poisoning.	*Primusquine Phosphate (equivalent of 60 mg of primaquine base)	For the prophylaxis of malaria, once a week, in association with a schizonticide.
*Caramphen Hydrochloride		Initial dose daily, in divided doses, subsequent doses increasing gradually to 600 mg daily in accordance with the needs of the patient.	(equivalent of 15 mg of primaquine base)	Daily for fourteen days, for the radical cure of <i>P. vivax</i> malaria.
*Cyclizine Hydrochloride		One to four times daily	*Progesterone	By intramuscular injection, daily
*Dichlorphenamide		By subcutaneous or intramuscular injection.	75 mg	
*Dipipanone Hydrochloride		Initial dose, increasing gradually to 500 mg daily in accordance with the needs of the patient.	Ascorbic Acid 500 mg	Daily prophylactic dose. Daily therapeutic dose.
*Ethopropazine Hydrochloride		Initial dose daily in divided doses, subsequent doses may be increased gradually to a maximum of 200 mg daily, in accordance with the needs of the patient.	*Diphenhydramine Hydrochloride 50 mg	By intramuscular or intravenous injection in single or divided doses.
*Hydralazine Hydrochloride			*Tolazoline Hydrochloride	
			80 mg	
			*Indigo Carmine	By intravenous injection.
			Mephenetermine Sulphate	By intramuscular or slow intravenous injection.
			(equivalent of 80 mg of mephenetermine base)	
			Methylprednisolone	Daily, in divided doses
			*Antazoline Hydrochloride	
			B.C.G. Vaccine	Prophylactic, by intracutaneous injection, as a single dose.

*Deoxycortone Tri-methylacetate	By intramuscular injection, once a month	Anise Oil Prepared Belladonna Herb
*Dimenhydrinate 50 mg	By intramuscular injection	*Butobarbitone
*Ethisterone Daily	Daily	*Chlorychzine Hydrochloride
*Hydrochlorothiazide Hydroflumethiazide Ipecacuanha Liquid Extract	Daily or on alternate days	Emetine and Bismuth Iodide
Mepacrine Hydro-chloride	Daily or on alternate days	*Ferrous Sulphate, Dried 1 2 G
500 mg	Suppressive dose daily, in the treatment of malaria.	*Mepyramine Maleate 50 mg
1 G	Therapeutic dose, daily, in divided doses	Mercaptoperine Nux Vomica Liquid Extract
*Methion	In divided doses in the treatment of tapeworm infestation	Powdered Opium
*Pethidine Hydro-chloride	Daily increasing to 600 mg in accordance with the needs of the patient	*Pentobarbitone Sodium
50 mg	Orally and by subcutaneous and intramuscular injection	Peppermint Oil
Phentolamine Hydro-chloride	By intravenous injection	*Quinalbarbitone Sodium
*Prednisolone	Daily, in divided doses	*Quinidine Sulphate
Prednisolone Acetate	Daily in divided doses	300 mg
*Prednisone	Daily, in divided doses	Santonin
Prednisone Acetate	Daily, in divided doses	Stramonium Liquid Extract
*Prochlorperazine Maleate	Daily in divided doses in psychiatric states	*Sulphamethizole
30 mg	As an antiemetic	240 mg Pyridostigmine Bromide
Sodium Anoxynaph-thonate	By intravenous injection	5 mg
Tripeleannamine Hydro-chloride		The frequency of the dose is determined in accordance with the needs of the patient.
120 mg		By intramuscular or subcutaneous injection The frequency of the dose being determined in accordance with the needs of the patient.
*Phenobarbitone		
*Phenobarbitone Sodium		
200 mg	By intravenous or intramuscular injection as a single dose	250 mg *Acetarsol
125 mg		Cascara Dry Extract
Prepared Ipecacuanha		*Phenoxymethypenicillin
150 mg		Phenoxymethypenicillin Calcium (equivalent of 250 mg phenoxymethypenicillin)
Diethazine Hydro-chloride	Initial dose, daily in divided doses subsequent doses increasing gradually to 1 5 G in accordance with the need of the patient.	*Phenoxymethypenicillin Potassium (equivalent of 250 mg phenoxymethypenicillin)
*Impramine Hydro-chloride	Daily in divided doses	Thyroid
*Nitrofurantoin	Four times daily	300 mg Aloes
200 mg or 0 2 ml	Orally and By intravenous intramuscular or subcutaneous injection the dose is determined by the physician in accordance with the needs of the patient.	*Amanophylline 500 mg
*Amylobarbitone		*Ferrous Gluconate 2-4 G
*Amylobarbitone Sodium		By slow intravenous injection
		Daily prophylactic dose
		Daily therapeutic in divided doses.

300 mg (continued)				
*Ferrous Sulphate 1 8 G	Daily prophylactic dose Daily therapeutic dose, in divided doses	1 2 G 200 mg 200 mg	Initial dose, daily Daily maintenance dose	
Pentamidine Isethionate	Prophylactic by intramuscular injection every three to six months, in the treatment of trypanosomiasis. Daily for seven to fifteen days, by intramuscular injection, in the treatment of early trypanosomiasis and leishmaniasis	Methyprylon Phenylbutazone 500 mg or 0.5 ml *Acetazolamide 250 mg	Daily in the treatment of giardiasis As a hypnotic Also as a sedative, daily in divided doses. Daily in divided doses	
Phanquone	Daily, in divided doses, for ten days	Adrenaline Injection 0 05 ml per minute	Initial dose. Subsequent doses every six hours.	
*Phenindione	Initial dose, subsequent doses 25 to 100 mg daily, in accordance with the prothrombin activity of the blood.	Benzylpenicillin 600 mg	Single dose by subcutaneous injection. By subcutaneous injection, in the treatment of status asthmaticus and other allergic emergencies. Every four hours.	
*Phenolphthalein *Proguanil Hydrochloride Subophen	Daily, as a suppressive of malaria By intramuscular or intravenous injection, daily or at longer intervals, to a total dose of 4 5 G	Caffeine Caffeine Hydrate Chloroquine Phosphate 1 G 500 mg	By intramuscular injection, two to twelve times daily	
400 mg	Amodiaquine Hydrochloride (equivalent of 400 mg of amodiaquine base) 600 mg (equivalent of 600 mg of amodiaquine base)	Equivalent of 300 mg of chloroquine base	In the treatment of malaria Suppressive dose, weekly Initial therapeutic doses, daily Adult therapeutic dose by intravenous or intramuscular injection	
*Chloroquine Sulphate	Therapeutic dose daily for 3 days In the treatment of malaria	1 5 G 250 mg 250 mg	In the treatment of discoid lupus erythematosus and rheumatoid arthritis Initial daily dose Daily maintenance dose	
800 mg 400 mg	Suppressive dose weekly Initial therapeutic dose Subsequent therapeutic doses, daily	1 G	Daily in the treatment of giardiasis	
(equivalent of 300 mg of chloroquine base)	Adult therapeutic dose by intravenous or intramuscular injection. In the treatment of discoid lupus erythematosus, and rheumatoid arthritis	*Chlorpromazine Hydrochloride 50 mg	Daily in the treatment of hepatic amoebiasis. Daily, in divided doses, in psychiatric states Orally and by intramuscular injection as an antiemetic	
1 2 G 200 mg 200 mg 800 mg	Initial dose daily Daily maintenance dose Daily in the treatment of giardiasis Daily in the treatment of hepatic amoebiasis.	*Chlorpropamide Diethylcarbamazine Citrate Strong Ginger Tincture Halibut liver Oil *Glutethimide Hyoscyamus Liquid Extract Heavy Magnesium Carbonate 5 G	Daily	
*Cortisone Acetate	Daily in divided doses, orally and by intramuscular injection Daily, for replacement therapy after adrenalectomy in Addison's disease, and in panhypopituitarism.	Light Magnesium Carbonate 5 G	As an antacid	
50 mg	In the treatment of malana	Light Magnesium Oxide 5 G	As a laxative	
Hydroxychloroquine Sulphate	Suppressive dose, weekly Initial therapeutic dose. Subsequent therapeutic doses, daily	Potassium Iodide 50 mg	As an antacid. As a laxative As an expectorant.	
800 mg 400 mg	In the treatment of discoid lupus erythematosus and rheumatoid arthritis.	*Primadone	In the pre-operative treatment of thyrotoxicosis. Daily, increasing to 2 G in accordance with the needs of the patient	

Sodium Iodide 50 mg	As an expectorant In the pre-operative treatment of thyrotoxicosis	300 mg	Child's dose, daily in divided doses, increasing to 900 mg in accordance with the needs of the patient.
*Streptomycin Sulphate (equivalent of 500 mg of streptomycin base) (equivalent of 1 G of streptomycin base)	Every eight hours as an intestinal antiseptic	1 G or 1 ml *Acetylsalicylic Acid 8 G	Daily, in divided doses, in the treatment of acute rheumatism.
Suramin 2 G	By intramuscular injection, daily or at longer intervals Initial dose by intravenous injection Subsequent doses, weekly for five weeks	Dried Aluminium Hydroxide Gel *Carbromal Ethotoin	Daily, increasing to 3 G in accordance with the needs of the patient
Thambutosine	Daily, increasing every fourteen days by 500 mg to a maximum of 3 G	Ginger Griseofulvin *Hexylresorcinol	Daily in divided doses In the treatment ascariasis of infestation
*Thiopentone Sodium 2 G	By intravenous injection Maximum rectal dose (40 mg per kg body weight)	100 mg Aqueous Iodine Solution Lucanthone Hydrochloride *Methicillin Sodium	As a single dose for adults Children's dose per year of age up to ten years In the pre-operative treatment of thyrotoxicosis
600 mg		*Paracetamol Concentrated Peppermint Water Rhubarb Sulphamethoxypudazine 500 mg *Ipecacuanha Tincture 20 ml	Twice a day for three days By intramuscular injection, every four hours. Initial dose Subsequent doses, daily As an emetic
*Barbitone Sodium *Ipecacuanha and Opium Powder *Isomiazid *Methylthioureas 200 mg Phenacetin Propylthiouracil 200 mg Quinine Bisulphate Quinine Dihydrochloride Quinine Hydrochloride *Quinine Sulphate *Testosterone Thiordazine Hydrochloride	Daily in divided doses. Controlling dose, daily Maintenance dose, daily Controlling dose, daily Maintenance dose, daily Orally and by intravenous injection Total implantation dose Daily in divided doses	Prepared Digitalis 200 mg Diloxandine Furatoate *Procainamide Hydrochloride *Tolbutamide	Daily in divided doses Initial dose, in divided doses, for rapid digitalization Maintenance dose daily Daily for ten days or more Also by slow intravenous injection of a 2.5 per cent w/v solution, of an amount determined by the effects produced Daily
750 mg *Cycloserine 800 mg Dimercaprol	Daily in divided doses By intramuscular injection, in divided doses on the first day, 400 mg in divided doses on the second and third days, and 200 mg in divided doses on subsequent days Controlling dose, daily, in divided doses Maintenance dose, daily Daily in divided doses, orally and by intramuscular and intravenous injection	1 2 G or 1 2 ml *Meprobamate 1 5 G	Daily in divided doses
*Potassium Perchlorate 400 mg *Promazine Hydrochloride	By intramuscular injection every two or three weeks as a prophylactic Adult dose, daily, in divided doses, increasing to 1 8 G in accordance with the needs of the patient Child's dose, daily, in divided doses, increasing to 900 mg in accordance with the needs of the patient By intramuscular injection, daily	1 8 G Demethylchlortetra cycline Hydrochloride 2 G or 2 ml *Belladonna Tincture *Chloral Hydrate Chloroform Spirit Chlorothiazide Colchicum Tincture Di iodohydroxyquinoline *Erythromycin Magnesium Trisilicate Mersalyl Injection	Daily or on alternate days Daily for twenty days Daily in divided doses By intramuscular injection, on alternate days.
900 mg Benzathine Penicillin  *Paramethadione	Adult dose, daily, in divided doses, increasing to 1 8 G in accordance with the needs of the patient	*Morphine Hydrochloride Soluton Nikethamide Novobiocin Calcium	By intravenous injection Daily, in divided doses
300 mg  *Procaine Penicillin  *Troxidone	Child's dose, daily, in divided doses, increasing to 900 mg in accordance with the needs of the patient By intramuscular injection, daily Adult dose, daily in divided doses, increasing to 1 8 G in accordance with the needs of the patient		

## DISPENSING FOR PHARMACEUTICAL STUDENTS

<b>2 G or 2 ml (continued)</b>			
Novobiocin Sodium (in each case the equivalent of 2 G of novobiocin)	Daily, in divided doses.	<b>4 G</b>	Daily, in divided doses, in the treatment of infections of the urinary tract.
*Nux Vomica Tincture		<b>*Tetrachlorethylene</b>	As a single dose.
*Opium Tincture		<b>*Tetracycline Hydrochloride</b>	Daily, in divided doses.
Orange Tincture		<b>2 G</b>	
*Oxytetracycline Hydrochloride	By intravenous infusion, in a concentration not exceeding 0·1 per cent w/v, daily	Tryparsamide	Daily, by intravenous infusion, in a concentration not exceeding 0·5 per cent w/v
*Piperazine Adipate	For all three piperazine salts —	Urethane	By subcutaneous, intramuscular or intravenous injection at intervals of five to seven days to a total of 24 G
*Piperazine Citrate	Adult dose, daily, in divided doses in the treatment of threadworm infestations.	<b>4 G or 4 ml</b>	Daily
*Piperazine Phosphate		Methylcellulose 450	
<b>4 5 G</b>	Adult single dose, as an ascariocide Doses for a child are calculated on body weight.	*Sodium Calcium-meditate	Daily, in divided doses. Daily, in divided doses, in accordance with the needs of the patient.
Potassium Citrate	Daily	Sodium Citrate	Also given by intravenous infusion, the dose being calculated on body weight
Probenecide		<b>5 G or 5 ml</b>	
Senna Fruit		Calcium Carbonate	
Powdered Senna Leaf		Calcium Gluconate	
Sodium Stibogluconate	By intramuscular or intravenous injection daily for ten to thirty days.	<b>2 G</b>	By intramuscular or intra-venous injection.
*Stramonium Tincture		Calcium Lactate	
*Sulphadimidine Sodium	By intramuscular or intravenous injection.	Compound Cardamom Tincture	
<b>3 G or 3 ml</b>		Cascara Elixir	
*Chloramphenicol	Adult dose, daily, in divided doses. Doses for a child are calculated on body weight.	Cascara Liquid Extract	
*Chlortetracycline Hydrochloride	Daily, in divided doses	Chalk	
Ferrous Ammonium Citrate		Diophthal	By enunction three times a week or 1 5 G daily
Weak Ginger Tincture		Compound Gentian Tincture	
*Oxytetracycline Di-hydrate	Daily, in divided doses	Concentrated Compound Gentian Infusion	
Pancreatin		Ginger Syrup	
*Selapsone	Daily Also given as an intramuscular injection, 0·25 ml of a 50 per cent w/v solution, twice a week, increasing to a maximum of 3 ml twice a week.	*Hyoscyamus Tincture	
Sulphadiazine	Initial dose, subsequent doses up to 4 G daily, in divided doses	Liquorice Liquid Extract	
*Sulphadimidine	Initial dose, in the treatment of systemic infections, subsequent doses up to 6 G daily, in divided doses.	Orange Syrup	
<b>2 G</b>	Initial dose, in the treatment of infections of the urinary tract, subsequent doses up to 4 G daily, in divided doses	Dilute Phosphoric Acid	
*Sulphafurazole	As for sulphadimidine.	Compound Rhubarb Tincture	
*Sulphasomudine	Initial dose, in the treatment of systemic infections, subsequent doses up to 6 G daily, in divided doses.	Sodium Bicarbonate	
		<b>6 G or 6 ml</b>	
		Ammonium Chloride	Daily, in divided doses, before the administration of Mersalyl Injection.
		Ipanoic Acid	As a single dose ten to fifteen hours before radiographic examination.
		Male Fern Extract	After fasting for twenty-four hours.
		Potassium Bromide	Daily, in divided doses
		Sodium Bromide	Daily, in divided doses.
		<b>8 ml</b>	
		*Paraldehyde	Orally and by intramuscular injection.
		<b>30 ml</b>	By rectal injection as a basal anaesthetic.

10 G or 10 ml Dilute Hydrochloric Acid		Calcium Amino- salicylate	Daily, in divided doses
Magnesium Hydroxide Mixture	As an antacid	Magnesium Sulphate	
50 ml *Camphorated Opium Tincture	As a laxative	Sodium Aminosalicylate	Daily, in divided doses
Phthalylsulphathiazole	Daily, in divided doses	Sodium Sulphate	
Sodium Salicylate	Daily, in divided doses, in the treatment of acute rheumatism	20 G or 20 ml Castor Oil	
Tolu Syrup		*Succinylsulphathiazole	Daily, in divided doses
15 G or 15 ml Aluminium Hydroxide Gel		Liquid Paraffin	
		40 ml Compound Gentian Infusion	
		75 G Light Kaolin	

## Appendix 8

### Answers to Calculations

#### Exercise 3.1

1 (a) Camphor	5 oz avoir	4 131 25	"	or 9 G in 360 ml Use 12 fluid oz.
Oil of Lavender	5 35 00	"		
Strong Solution of Ammonia	96 minims	6 35-00	"	
	11 fluid oz	7 0 875	"	or 60 mg in 600 ml Use 1 pint
	120 minims	8 21 875	"	or 1 5 G in 240 ml Use 8 fluid oz.
Alcohol (95%), sufficient to produce	9 6 5625	"		or 0 45 G in 360 ml Use 12 fluid oz.
(b) Oil of Peppermint	40 fluid oz	10 17 50	"	
Alcohol (90%)	96 minims	11 4 375	"	or 0 3 G in 240 ml Use 8 fluid oz.
Distilled Water, sufficient to produce	6 fluid oz	12 78 75	"	or 5 4 G in 600 ml Use 1 pint
The quantity of talc is $\frac{1}{2}$ oz avoir	10 fluid oz	13 (a) 8 tablets		
		(b) 1 "		
		(c) 2 "		

2 (a) Calamine	3 oz		
Zinc Oxide	1 oz		
Bentonite	262 5 gr		
Sodium Citrate	43 75(44) gr		
Liquefied Phenol	48 minims	1 112 00 grains	
Glycerin	1 fluid oz	2 746 66	"
Purified Water, sufficient to produce	20 fluid oz	3 280 00	"
(b) Wool Alcohols	52 5 gr	4 420 00	"
Hard Paraffin	210 gr	5 560 00	"
White or Yellow Soft Paraffin	87 5 gr	6 116 66	"
Liquid Paraffin	525 gr	7 14 00	"
		8 350 00	"
		9 84 00	"
		10 42 00	"

#### Exercise 3.2

1 Atropine Sulphate	1½ gr		
Water, to produce	165 minims		
or Atropine Sulphate	0 1 G		
Water, to produce	10·0 ml		
2 Mercury Oxycyanide	1 gr		
Water, to produce	110 minims		
Dilute 10 minims of this solution to 500 minims to produce			
1 in 5,000			
or Mercury Oxycyanide	50 mg		
Water, to produce	50 ml		
Dilute 6 ml to 30 ml to produce a 1 in 5,000 solution			
3 Acenflavine	1 gr		
Water, to produce	550 minims		
Mix $\frac{1}{2}$ oz of this solution with $\frac{1}{2}$ oz alcohol (90%) and add			
water to produce 1 fluid oz (see note on p. 31 regarding			
the contraction which takes place when alcohol is diluted			
with water)			

#### Exercise 3.3

1. 0·875 grains or 60 mg in 120 ml. Use 4 fluid oz.
2. 78 75, " or 5 4 G in 180 ml. Use 6 fluid oz.
3. 140·00, "

#### Exercise 3.4

	1 112 00 grains
	2 746 66
	3 280 00
	4 420 00
	5 560 00
	6 116 66
	7 14 00
	8 350 00
	9 84 00
	10 42 00

#### Exercise 3.5

1	140 gr and 1 tablespoonful to be diluted to a quart
2	65 625 gr and 1 tablespoonful to be diluted to a pint
3	1,400 gr and 2 tablespoonsfuls to be diluted to 2 gallons
4	525 gr and 2 teaspoonsfuls to be diluted to 30 oz
5	1 680 gr and 8 tablespoonsfuls to be diluted to 1 gallon
6	1 050 gr and 2 tablespoonsfuls to be diluted to half a gallon
7	60 minims
8	1 oz 288 minims

#### Exercise 3.6

1	8 oz 426 minims
2	466 6 ml
3	3 oz 160 minims
4	111 1 ml
5	20 oz
6	266 6 ml
7	4 oz 320 minims
8	5 oz 160 minims
9	53 oz 160 minims
10	1,500 ml

**Exercise 37**

1 As shown above for Exercise 31 (a) and (b) respectively	4 2 100 grains
2 Sodium Bicarbonate	5 87 5 grains
Borax	6 175 grains
Glycerin	7 87 5 grains
Water sufficient to produce	8 216 minims diluted to 3 fluid oz
3 Sodium Bicarbonate	9 21 875 grains
Borax	10 175 grains tannic acid 8 75 grains mercuric chloride
Glycerin	11 21 875 grains or 1 5 G in 30 ml Use 1 fluid oz
Water sufficient to produce	12 2 187 5 grains alum 1 750 grains zinc sulphate
4 Menthol	13 58 3 grains or 4 G in 600 ml Use 1 pint
Camphor	14 52 5 grains
Thymol	15 3 oz 96 minims
Oil of Eucalyptus	16 1 oz (avoir weight) 357 9 gr diluted to 4 oz (avoir weight)
Liquid Paraffin sufficient to produce	17 8 75 grains or 0 6 G in 300 ml Use 10 fluid oz

**Exercise 38**

1 3 oz (avoir) 13 gr to be diluted to 20 oz (avoir) by weight	21 48 minims
2 2 oz (avoir) 185 gr to be diluted to 4 oz (avoir) by weight	22 175 grains
3 113 636 G to be diluted to 250 G	23 144 minims
4 215 gr to be diluted to 8 oz (avoir) by weight	24 155 5 millilitres
5 96 minims to be diluted to 2 fluid oz	25 8 oz
6 4 oz 49 minims to be diluted to half a gallon	26 Zinc chloride 0 625 grains in 550 minims or 375 mg in 30 ml

**Exercise 39**

1 840 grains	27 [The answers to these questions may be checked from the
2 35 grains	28 formula (Imperial system) given in the B P C
3 17 5 grains	29 Atropine Sulphate 1 $\frac{1}{2}$ gr Water to 330 minims or 50 mg in 10 ml

# Index

Note References in bold type are of particular importance  
Illustrations are indicated by *italic type*

A B P I 9, 114, 531, 532

Absorption

liquid, by filters 366, 479, 483  
ultra-violet light 527, 528

Acacia

binder in compressed tablets, 101  
emulsions 56-61  
protective colloid 51

Accelerator

travelling wave linear 530, 533  
Van de Graaff 530

Accuracy

dispensing 3, 21, 354  
labelling 356

Adeps (Lard) 133, 156, 159

Adeps Lanae (Wool Fat) 136, 159

Adeps Lanae Hydrosus (Hydrous Wool Fat) 136, 159

Adrenaline

in local anaesthetics 277  
instability 265, 268, 389, 391, 482

Adrenaline Injection

instability 268, 277, 294, 549  
mould growth in 234

preparation 355, 356, 391-2

Adrenaline Solution 284, 388

Adsorption

bactericides on bacteria 455, 456  
inhibitors by bacteria proof filters 474, 475

medicament by bacteria proof filters 457, 481, 482, 483, 493

medicament by charcoal 364

medicament by clarifying filters 365, 366, 368

pyrogen removal by 364

Aerobes 223, 410, 436, 448, 449

Aerosol, bacterial 416, 419

Aerosol producer 404, 416, 418

Agar

emulsions 60  
in culture media 232, 234, 449, 450, 465, 471

plates, mixing 473

plates, pouring 473, 474

radiation penetration studies with 532

Air

asepsis laboratory 409-21, 450, 469, 470

bacterial flora 400-1, 416, 523

boiler steam, in 323

break 333

Air—(contd)

centrifuge 418  
changes 421, 529  
compressed  
asepsis laboratory 405  
use for bacterial filtration 485  
use for filter testing 491  
use in quick cooling autoclaves 337

cooling of ultra violet tubes 528

density 329

drying of dressings with 334

effect on steam temperature 322, 323

fermenter 410, 413, 415

films in sterilisation 324, 331, 541

filtration, *see* Filtration

heating agent 311, 319

humidity control 410

injection into multi-dose containers 258

insulating effect 312, 315, 316, 324

lock 403, 407

mixed with steam 318, 322-4, 329, 546

movements

contamination from 401, 402, 403, 406, 408, 423

reduction of 422, 426

pockets in

containers 323, 338, 341, 540

hot air oven 311

steam sterilisers 323, 324, 329, 328, 329, 331, 332, 335, 337, 546

recirculation 410

removal

containers 338, 340, 341, 541

ethylene oxide sterilisation 521

large sterilisers 323, 324, 329, 440

by double vacuum 331, 342

by downward displacement 329-30, 331, 335-6, 339, 421

with turbulence 335, 336

by high vacuum 331 2, 336, 339, 342

by low vacuum 330-1

portable sterilisers 322, 325, 326, 328

replacement in injections 267, 268, 276, 292, 314, 392, 393, 404, 503,

562, 563

sampling, *see* Sampling

specific heat 311

Air—(contd)

sterilisation, *see* Sterilisation

stratification 323, 324, 335

temperature control 404, 410

velocity in air sterilisation 411, 412, 420, 528

Airing in gaseous sterilisation 520, 524

Alcohol (*see also* Ethyl alcohol) dilutions 2

Alcohols

filtration sterilisation of solutions 482, 494

inactivation in sterility testing 454, 467

injection vehicles 244

Algic acid

disintegrating agent 105

emulsifying agent 56, 63

Alkalinity of glass 266, 274-7, 279, 300, 316

Alligation 37, 38

proof of 39

Alkaloid instability 265, 276, 283, 482

Alkaloidal salt instability 276, 317

Alpha particles 529, 530

Aluminum hydroxide

in lotions 127

in pastes 152

Aluminum stearate, gelling agent 271, 314

Amaranth 371, 379

Amethocaine Injection 239, 277

Amunacrine dusting powder 315

Amunophylline instability 243, 265, 268

Ammoniated mercury ointments 346

Ampoule

amber 392

annealing 376

aseptic filling 430-1

aseptic filling 436

aseptic opening 430-2

aseptic sealing 436

aseptic transference from 430

block 436

charring 375, 377, 378

crack detection 361, 371

cutters 375, 376, 429

drying 376

easily-opened 297

filling devices 375

filling 306, 348, 394

& hypodermic tablets 545

**Ampoule—(contd)**  
 inspection for particles 371  
 keeping neck dry 393  
 labelling 356-61, 380, 498, 499  
 liquid 297  
 list of injections requiring 246, 260,  
 292, 314, 393, 515, 563  
 manipulation volumes 374  
 minimum size 392  
 neck length 375, 390  
 neutral tubing for 279  
 opening 306, 375, 376  
 powder 297, 501-2  
 preparation for asepsis 435  
 preparation for use 375  
 protection of mouth 430  
 seal testing 371-2, 380, 387  
 sealing 375, 378-9  
 sealing faults 379  
 solids for injection, for 277, 499  
 solvents in 514, 505  
 spicules from 278, 514  
 splintering 376  
 sterilisation 312, 349  
 of outside of 361, 430, 523  
 sterility test sample volumes 462  
 test for alkalinity 281, 282  
 thermal resistance 279  
 thickness 278  
 tube 297  
 visibility of contents 357  
 volume tolerances 370, 374  
 washing devices 376, 377  
 weight tolerances 501

**Anaerobes** 223, 228, 448, 449, 450, 471  
 facultative 223

**Anaesthetics**

for injection pain 246, 349

intrathecal 239

intravenous 237

local 259, 266, 277

spinal 269, 361

**Aneurnine, bacterial growth factor** 223

**Aneurine Injection** 277, 400, 459, 482,  
 494-8

**Angstrom unit** 526

**Animal products\***

pyrogen in 364, 556

sterilisation 490, 492

**Anticoagulants** 247, 278

**Antigenicity, loss of** 261, 269, 346,  
 529, 533

**Antibiotics**

dermatitis from 409

incompatibilities with bactericides  
 557

instability 265, 267

preparation of injections 505

pyrogen in 364, 365, 555

radiation sterilisation 532

sterility test 446, 463

**Antiseptics** 156, 549

in emulsions 70

in injections 268, 294, 349, 354, 386

in ointments 156

in rubber 289

in wool alcohols 156

**Antiseptic solution for Clenbuterol liners**  
 304, 305, 385

**Apomorphine Injection** instability  
 268, 276, 392

**Apomorphine instability** 268, 393, 482

**Apothecaries system of weights** 9  
 declining use of 9-11

**Applications** 122  
 B P and B P C. 131

**Applications of**

Benzyl Benzoate 131

Calamine, Compound 131

Dicophane 131

Gamma Benzene Hexachloride 131

Salicylic Acid and Sulphur 131

**Approximations in dispensing** 22 (Ex  
 3 13)

**Aqueous Cream (Hydrous Emulsifying  
 Ointment)** 133, 139, 143

**Aqueous Ointment (Oily Cream)** 137,  
 160

**Arachis oil, injection vehicle** 244, 245,  
 271, 314

**Arctone** 517, 521

**Aromatic waters** 43

**Arsenicals, sterility test** 446, 455

**Asphenamates, oxygen sensitivity**  
 505

**Ascorbic acid**

protectant from radiation damage  
 533

reducing agent in injections 267,  
 268

**Ascorbic Acid Injection**, instability  
 263, 268, 277, 350

**Aseptic technique** 309, 314, 400-43,  
 450, 498, 523

arrangement of working area 424,  
 425

clothing 450

disturbance during 402, 426

equipment for 312, 422

exercises in 421-43, 450

fitting syringe needle 434

handling 433, 499, 519, 533

injection preparation 464, 559

liquid dilution 442

pouring 442

preparation for working 422, 450

preparation of penicillin preparations  
 512, 513-5

preparation of solutions 443

reduction of contamination sources  
 421-2, 426

removal of paper caps 435

sealing bottles 440

sterilisation by filtration 446, 479-  
 98

Tyndallisation, in 348

ultra violet light as aid 529

vaccine production 347

washing plastic containers 350

weighing 443

**Atmosphere, contamination source**  
 400-1, 422

**Atomiser for bacterial aerosols** 417

**Atropine Eye Ointment, preparation**  
 509

**Atropine Injection, instability** 276

**Auricularia** 168

**Autoclave**

damage to plastic surfaces 404

opening 278, 382

**Portable** 325

for ethylene oxide sterilisation  
 521

pressure controlled 325-6, 328,

329

**Sphinx** 328

temperature controlled 326-8

T R F 326, 327, 328

release of pressure 278

**Autoclaving, see Heating in an Auto-**

**clave**

**Automatic control of sterilisation proc-**

**esses** 312, 337, 338, 347, 520, 533

**Autoxidation** 550

**Bacillus cereus**, penicillase from 456

**Bacillus pumilus**, radiation test organism  
 531

**Bacillus stearothermophilus**, moist heat

resistance 224, 344

**Bacillus subtilis** 343, 419, 518, 521

aerosol 420

penicillase from 456

*tau globulin* 518, 521

**Bacitracin, sterility test** 458, 475

**Bacteria** 222, 493

aerobic, *see Aerobes*

air, in 400-1, 416, 418, 469

anaerobic, *see Anaerobes*

cell membrane 456

chromogenic 491

colonies 419, 473, 485

commensal, *see Commensals*

damage to medicaments 259

damaged 447, 449, 451, 527

drying of 344, 419, 467, 520

essential metabolites, *see Essential*

metabolites

ethylene oxide on 518

exacting 348, 449, 453, 465, 467

factors affecting growth 223-4

filter passing 493

growth curve 225, 454

growth phases 225-6

healthy 447, 449

humidifiers, in 410

ionising radiations on 531

lethal heat exposures 309, 316

mesophilic, *see Mesophiles*

metabolic reactivation 527

multiplication in injections 366, 396

multiplication in water 364

nutrition 223, 226-7, 449, 450

optimum conditions for growth  
 223-4, 447

pathogenic, *see Pathogens*

penicillin resistant 305

penicillase producers 513

photoreactivation 527

psychotrophic, *see Psychotrophs*

revival 453, 455, 456

size 401, 491

skin flora 401-2, 424, 426

spores, *see Spores*

## INDEX

Bacteria—(contd)  
 streptomycin sensitive 458  
 thermophilic, *see* Thermophiles  
 toxicity of rubber extracts to 292,  
 296  
 transferring suspensions 429  
 ultra violet resistance 527  
 wet 344, 467

Bactericides  
 absorption by injection closures 259, 293–4  
 acid injections, in 277  
 activity spectrum 423, 424  
 aerial 411, 415–18, 420  
 aqueous preparations, for 259  
 as injection preservatives 224, 258–  
 61, 349, 374  
 bonding by plastics 285, 286, 536  
 catgut tubing fluids 467, 469  
 damage to equipment 517  
 definition 224  
 desirable features 259, 348  
 detergent 423  
 diffusion through rubber 293, 304  
 dilution coefficient 454  
 effect of organic matter on 426  
 effect of temperature on 317  
 equilibration with closures 293, 294  
 impurities in 557  
 in filtration sterilised injections 259,  
 493, 494  
 in Heating with a Bactericide 224,  
 374  
 in infusion fluids 364, 380  
 in injections 258–61, 348, 354  
 in injections assayed by light absorp-  
 tion 260  
 in intracisternal injections 260, 389  
 in intrathecal injections 260, 389,  
 515  
 in intravenous injections 239, 260,  
 300, 389  
 in multi dose containers 258, 265,  
 317, 349, 382, 494  
 in oily preparations 259–60, 350, 391  
 in penicillin preparations 457, 513,  
 514, 515  
 in peridural injections 260, 389  
 in Water for Injection 260  
 incompatibilities of 259, 261, 348,  
 350, 354, 382, 424, 425, 557  
 influence on sterility testing 453,  
 459, 474, 496  
 Labelling requirements 357–61  
 loss from closures 293–4, 295  
 omission from injections 265, 317,  
 394, 563  
 permeability of plastics to 285  
 persistence of effect 423, 424, 425  
 pH stability 265, 317, 348  
 sporcidial 349  
 staining 424  
 storage stability 348  
 surface 524  
 toxicity 259, 348, 361, 423, 424  
 turbidity from 261  
 vaccine sterilisation for 346  
 Bacteriological techniques, separate  
 room for 427

Bacteriostats 224  
 Bag  
 muslin, for ampoule sterilisation 372, 380, 387  
 paper, heat sealed 338, 341, 361,  
 490  
 plastic, for injections 303  
 Baffle  
 ethylene oxide steriliser 520  
 phantomiser 416, 417  
 steam separator 332  
 steam steriliser 329  
 still head 241  
 Balance 3, 354, 523  
 for asepsis 407, 443  
 Balsam of Peru, in suppositories 176  
 Barbiturates  
 incompatibilities 211, 212  
 instability 268, 505  
 sterility test 454, 464, 502  
 Barrier creams 144, 145  
 Bases, instability of synthetic 265, 277  
 Bassorin Paste 151  
 Beaker  
 as sterile cover 434, 501, 505  
 covered for Heating with a Bacteri-  
 cide 349, 387  
 sterilisation 313  
 Beeswax  
 in ointments 137, 138  
 in suppositories 166  
 sterilisation 313  
 sterility testing of ointments con-  
 taining 466  
 Benemegride Injection, labelling 260  
 Benches, asepsis laboratory 405, 406  
 Bentonite  
 in lotions 127  
 in pastes 152, 153  
 Benzalkonium chloride, in eye drops 188  
 inactivation in sterility testing 456  
 Benzathine penicillin, sterility test 456  
 Benzoic acid  
 antifungal activity 69, 235  
 binding by nylon 286  
 Benzoinated Lard 156, 159  
 Benzyl alcohol  
 absorption by rubber 293  
 anaesthetic activity 246, 259, 349,  
 394  
 antifungal activity 235  
 inactivation in sterility tests 459  
 injection bactericide 259, 349, 394  
 vehicle in oily injections 245  
 Benzyl benzoate, injection vehicle 245, 287  
 Benzyl Benzoate Application 131  
 Benzylpenicillin, *see* Penicillin  
 Beta particles 529  
 Beta propiolactone 517, 524  
 Binder, compressed tablets 101  
 Bismuth Injection, reducing agent in 550  
 Bismuth Oxychloride Injection 360,  
 393, 463  
 Bismuth Sodium Tartrate Injection, pH  
 adjustment 277, 366  
 Blankets, sterilisation 517, 519, 523  
 Blood  
 bottle, *see* Containers, Transfusion  
 bottle  
 glass flakes in 278  
 mixing with agar 474  
 oxygenator, sterilisation 520, 523  
 plasma  
 buffering power 265  
 freezing point 250, 253  
 osmotic pressure 250  
 plastic bag for 286, 287  
 products 446, 484, 490, 492  
 red cells, *see* Erythrocytes  
 removal of clots from 301  
 sterility test 446  
 survival of organisms in deposits 317, 519, 537  
 Boat, weighing 394, 443, 495, 501  
 Boiler, steam 318, 320, 323  
 Boiling, sterilisation by 317, 484, 541,  
 546  
 Bottled fluids, sterilisation 335–7,  
 338–9, 345  
 Bottles  
 culture media 229  
 McCartney 488  
 plastic, sterilisation 350, 523  
 rubber capped, ethylene oxide sterili-  
 sation 522  
 screw-capped, sterilisation of jellies  
 etc 546  
 Bougie  
 filling of mould 172  
 nasal 168  
 urethral 168  
 Bourdillon slit sampler 418, 419  
 Box  
 ampoule 357, 359, 360, 373, 392  
 light protection with 284, 297, 373,  
 385, 392  
 membrane incubation 484  
 rubber glove sterilisation 544  
 Breakages  
 in hot air oven 312  
 in steam sterilisation 326, 336, 337,  
 382, 396  
 Breath, as contamination source 401,  
 406, 422  
 Bremsstrahlung 533  
 British Thermal Unit (B T U) 319  
 Bronchoscope, sterilisation 523  
 Browne's tubes 345, 542  
 Brownian movement  
 bacterial aerosols 416  
 dust in air 401  
 in air filters 411  
 Bubble pressure 490, 492  
 Bubblers 419  
 Buccal tablets 97  
 Buffers in  
 creams 145  
 eye drops 192, 515  
 injections 265, 266–7, 277, 357, 383,  
 384, 512, 513, 514, 562  
 penicillin preparations 512–15  
 Buginaria 168  
 Bungs  
 particles from rubber 438  
 sticking of rubber 439

- Burette.  
for aseptic filling 407, 436, 437,  
438, 487  
for pH adjustment 395
- Burner, Bunsen  
in asepsis 404, 405, 410, 421, 422,  
423, 424  
Twin jet 370, 378
- Butylated hydroxy anisole 156, 551  
Butylated hydroxy toluene 156, 552  
By pass, steam steriliser 330, 335, 336,  
337
- CACHETS 83  
<sup>137</sup>Caesium 530  
Calamine Lotion 127  
Oily 17, 130  
Calciferol, in emulsions 59  
Calcium chloride  
deliquescence 397  
tower 369  
Calcium Gluconate Injection  
crystallisation 563  
precipitation in sterility test 459  
preparation 269, 366, 393  
pyrogens in 364  
Calcium lactate mixture 49  
Calcium d-saccharate, as stabiliser  
269, 393  
Calculations 15  
alcohol dilutions 32  
alligation 37  
concentrated solutions 23-7  
for injections 355  
for practise 397  
high dilutions 21  
isotonic solutions 250-8, 264  
milliequivalent 261-5  
percentage  
miscellaneous 33  
volume in volume 33  
weight in volume 17  
weight in volume and volume 19  
volume combined 33  
weight in weight 28  
proportional 37  
Camphorated oil 129  
*Candida albicans* 476  
Cannula 392, 439, 489, 513  
Cap  
aluminium for  
ampoule mouth 430  
glassware 312, 313  
transfusion bottle 300-1, 381  
tubes 229, 313, 384, 429, 431  
aluminium foil for  
needle containers 540, 541  
petrolatum powders 508  
syringe tubes 540, 541  
aseptic holding 427, 429, 434, 435  
bakelite Climbrite 304, 363, 385  
for hair in asepsis 408, 422  
metal screw with liner 229, 312,  
326, 338, 432, 462, 464, 523  
Oxid 229  
modified for weighing 443, 502  
paper for  
burettes 437
- Cap, paper for—(contd.)  
containers 313, 338, 363, 381,  
429, 435, 442, 472, 507  
delivery tubes 439  
filtration units 488  
syringe containers 433, 539, 440,  
541  
tubes 429, 435  
plastic overset 372, 508  
plastic screw with liner 338  
powder funnel 502  
rubber, see Closures  
tamper proof 372  
Capillary pipenger 419  
Capillitor  
for culture media 228  
for injections 266, 366, 395, 397  
Capping, multi dose containers 385  
Capsules  
enteric (as for pills) 95  
flexible gelatin 196  
hard 84  
manufacture 197  
oral depot 117  
seamless 197  
soft 196  
Caramelisation  
in culture media 546  
of dextrose 381  
Carbachol Injection, dextrose in 268,  
550  
Carbon dioxide 217  
diluent for ethylene oxide 517  
inert gas for injections 268  
saturation with 269  
Carbon tetrachloride  
for filter testing 491, 492  
solvent for syringe lubricants 539  
Carbohydrates, protection of organisms  
by 317  
Carbowaxes (Macrogols) 161, 167  
Cartridge 292, 297, 298, 393, 498, 499  
Cataplasma Kaolin 162  
Catechol, as antioxidant 551, 552  
Catgut 467-8, 545  
containers 467, 545  
heat sterilisation 467-8  
iodine sterilisation 467-8  
radiation sterilisation 467, 468, 544  
sterility test 466, 467-8  
testing tube seals 468  
Catheters, sterilisation of plastic 523  
524, 533  
Cationic emulsifying agents 56, 139  
144  
Cavitation 537, 538  
Cellulose derivatives  
in emulsions 62  
in pastes 151  
Cellulose acetate phthalate, as enteric  
coating 95  
Cements, heat resistance 313, 480  
Centrifugation, oil sterility tests 466  
Centrifuge  
method of bacterial filtration 488  
Wells air 418  
Cerebrospinal fluid 238  
bacterial growth in 260  
osmotic pressure disturbance 249  
Cerebrospinal fluid—(contd.)  
specific gravity 269  
volume 249, 250  
withdrawal of 239  
Cereols (arethral bougies) 168  
Cetomacrogol  
Emulsifying Ointment 141, 160, 161  
Emulsifying Wax 141  
Cetrimide  
bactericide in penicillin injections  
515  
Cream B N F 144  
Emulsifying Ointment 143, 160, 161  
Emulsifying Wax 144, 160, 161  
for swabbing surfaces 422, 423, 432  
in eye drops 189  
in sterility tests 456  
inactivation by soaps 425  
Chair, for asepsis 422, 423, 424  
Charcoal, air filter 413  
for pyrogen removal 364  
Charring  
in dry heat sterilisation 312, 313  
of ampoules 375, 377, 378  
of powders 502  
Checking  
balance 354  
doses 354  
injection calculations 355  
labels 356, 365  
medicaments 365  
poisons 3  
powder ampoule seals 502  
sediments in aqueous suspensions  
393  
volumes aseptically 442  
weights 356, 365, 384  
weights in powder ampoules 402  
Chlorbutanol  
antifungal activity 235  
in eye drops 189  
inactivation in sterility tests 454,  
459  
Chlororesol 355, 382, 386, 393  
absorption by rubber 293, 383  
antifungal activity 235, 249  
effect of organic matter on 261  
in emulsions 69  
in eye drops 189  
in Heating with a Bactericide 348,  
349, 388  
in multi-dose injections 259, 494  
in oily injections 260  
inactivation in sterility tests 454,  
459, 496  
incompatibilities 350, 557  
sterilisation of plastic containers  
350  
stock solution 383, 387  
toxicity 348  
volatility in steam 387  
Chlorhexidine, skin disinfectant 425  
for swabbing surfaces 423  
Chloroform, as preservative 69  
Chloroxylenol, for Climbrite liners  
304  
for swabbing surfaces 423  
Chlorpromazine, instability 268  
Chrononic gonadotrophin 364, 499

## INDEX

Chorionic Gonadotrophin Injection, preparation 277  
 Chrome acid, cleaning fluid 242, 280, 313, 362, 363, 481, 483  
*Chromobacterium prodigiosum* 491  
 Cinchocaine Hydrochloride Injection, specific gravity 270  
 Clarification 404, 492  
 of oils 390  
 Claying, of suspensions 270, 271  
 Cleaning  
 asepsis laboratory 402, 403, 404, 405, 406, 407-8, 423  
 dust from 401  
 equipment 318  
 filters 367, 481, 482, 483, 493  
 filtration units 488  
 floors 407  
 giving sets 301  
 glassware 312, 313, 361-3, 538, 562  
 injection containers 280 299, 317, 318, 361-3  
 instruments 543-4  
 needles 538  
 rubber gloves 544  
 syringe containers 539  
 syringes 317, 535, 537-8  
 tubing 317  
 Cleaning agents 362, 403, 405, 423  
 for closures 293, 363  
 for filters 481, 482, 483  
 Cleanliness 3  
 glassware 354  
 preparation of injections 349 354  
 labels 356  
 Clips  
 gate 369, 422, 439  
 spring 369, 437  
 Closing aseptic 479, 529  
 eye ointment tubes 511  
 tool for Clinbritic sealing rings 305, 370, 385  
*Clostridium spp.*, skin disinfectants on 426  
*Clostridium histolyticum*, sterility test control organism 460, 463  
*Clostridium sporogenes* radiation resistance 531  
 sterility test control organism 463, 471  
*Clostridium tetani* 223  
 heat resistance 310, 316, 317, 344  
 in air 400  
 in catgut 467  
 in powders 314  
*Clostridium welchu*  
 heat resistance 317  
 in air 400  
 in powders 314  
 Closures, for injections 288-96  
 cleaning 293, 294, 363-4  
 Clinbritic Bakelite 287  
 hypodermic solution tablets 545  
 penicillin preparations 512  
 rubber  
 absorption of bactericides 293  
 absorption of ethylene oxide 520  
 absorption of metabisulphite 294

Closures, rubber—(cont'd)  
 acid or alkalis from 266, 288, 295, 363  
 ageing 291, 296  
 antibiotic vial, disc type 306  
 antibiotic vial, plug type 305  
 bloom on 292  
 B P requirements 294  
 B S tests for 294-6  
 cartridge 297  
*Clinbritic Mark I*, skirted cap 291, 294, 304, 305, 385, 409, 439, 440, 441  
*Clinbritic Mark II*, plug 305, 306, 385, 439  
 colour from 292, 294, 296, 363  
 compatibility with contents 292, 294, 295  
 drying 364  
 elasticity 301  
 equilibration with bactericides 293-4, 304, 338, 364, 385, 496  
 ethylene oxide sterilisation 523  
 exclusion of micro-organisms by 306  
 extraction from injections 292  
 extractives from 292, 294, 296, 304, 306, 395  
 for microbiological use 292, 296  
 fragmentation 291, 293, 295, 303, 306  
 gas permeability 291, 292  
 hardness 296  
 heat resistance 391  
 inactivation of injections 292, 294, 295  
 inspection 364  
 loss of bactericide  
 by diffusion 293, 294  
 by volatilisation 293, 294  
 manufacture 290  
 moisture permeability 291  
 mould lubricant on 289, 292, 294, 363  
 multi dose container 258, 370  
 needle blockage 293  
 nikethamide attack on 563  
 oil resistant 291, 294, 391  
 particles from 294, 363, 394, 563  
 penetrability 291, 293, 294, 295, 306  
 penicillin vials 513  
 preparation of 267  
 protection from light 291, 305  
 quality 294, 295  
 re use 364  
 rinsing before use 382  
 sealing against bactericide loss 293  
 self sealability 291, 292, 294, 295, 303, 306  
 sterilisation 291, 338, 524  
 storage 291, 363  
 surface area 293  
 thickness 292, 293  
 toxic substances from 291, 292, 295, 296  
 Closures, rubber—(cont'd)  
 transfusion bottle 291, 296, 300, 301, 382  
 disc type 291, 301  
 plug type 291, 300, 381, 433, 444  
 turbidity from 292, 295  
 water vapour permeability 292, 296  
 zinc from 292, 363  
 Clothing  
 contaminants from 402, 408, 413, 422  
 for complicated aseptic techniques 405, 406, 409, 422  
 for preparation of injections 354  
 for protection from ultra violet 529  
 for simple aseptic techniques 408, 422  
 sterilisation of 339  
 Cloths for dressing packs 340  
 ethylene oxide permeability 519  
 Coating  
 of pills 94  
 of tablets 115  
<sup>60</sup>Cobalt 527, 530  
 Cocaine hydrochloride, isotonic solution 252, 254  
 Cocoa butter (*Theobroma cacao*) suppository base 166  
 Cod liver oil emulsions 58  
 Code, draft on the Dispensing of Proprietary 215  
 Cold cream 138  
 Collapsible tubes 134  
 Colloid, hydrophilic, suspending agent in injections 270  
 Colour, changes from ionising radiations 532  
 permitted food 371  
 Comfort  
 chairs 406  
 clothes 408  
 effect of humidity on 410  
 floorings 403  
 rubber gloves 409  
 Commensals 222  
 Comparator  
 for culture media 227  
 for injections 266  
 Competitive inhibition 455  
 Compound Powder of  
 Bismuth 91  
 Kaolin 92  
 Liquorice 92  
 Magnesium Carbonate 92  
 Rhubarb 92  
 Tragacanth, as suspending agent 45, 46  
 Compound powders of the B P C 91  
 Compressed tablets 97-121  
 abrasion tests 114  
 assay 109  
 binders 101, 102  
 buccal 97  
 chewing 97  
 coating 115  
 colouring 121

- Compressed tablets—(contd.)  
 controlled release of medicament  
 117  
 diameter 114  
 diluents 102  
 disintegrating agents 102  
 disintegration 110  
 dry extracts in 99  
 Dry Granulation 98  
 dynamic tests 118  
 enteric coated (as for pills), 95  
 tests for 115  
 formulation 102  
 granulation 98  
 Granulation by Preliminary Compression 98, 101  
 hand machine 105, 106  
 hygroscopic substances in 99  
 hypodermic 105, 545  
 implants 97, 474, 545–6  
 invention 97  
 lozenge 97, 104, 110  
 lubricants 101  
 machines 105, 106, 107, 108  
 mastication type 97, 110  
 mechanical strength 113, 114  
 Moist Granulation 98, 99  
 alternative method 99  
 official standards 109  
 official summaries 120, 121  
 oral 97, 110  
 oral depot 117  
 pan coating 99, 115, 116  
 penetration tests 114  
 preparation 98  
 large scale 100  
 press coating 116  
 rate of disintegration 110  
 recent advances 118  
 rotary machines 107, 108  
 shape 109 116  
 sieves for 98, 99  
 solution types 97, 103, 110  
 standards 109  
 static tests 118  
 storage 121  
 strength 113, 114  
 sublingual 97, 110  
 sugar coating 115, 116, 121  
 summaries of official 120, 121  
 volatile oils in 99  
 weight 109
- Compression, heavy, of implants 474  
 Concentrated infusions 43  
 Concentrated solutions, calculations  
 23–7  
 Condensate  
 damage to powders & oils 342  
 effect on vacuum pumps 332  
 from heating up 324, 334  
 removal from sterilisers 324, 330,  
 332–3, 334, 335, 336–7, 342  
 soaking of dressings 320, 329, 332  
 Condensation  
 in petri dishes 473  
 in steriliser jacket 332  
 in sterilisers 320  
 of saturated steam 319  
 on tiled surfaces 404
- Conduction, see Heat transfer  
 Coniferylbenzoate, as antioxidant 156  
 Conjunctivitis, from ultra violet 528  
 Container  
 cachet 83  
 capsule 84, 196  
 caigut 467, 468, 545  
 cream 134  
 culture medium 228, 230, 465, 473,  
 503  
 dusting powder 89, 315  
 dust proof 365, 422  
 ear drops 194  
 effervescent preparations 88  
 emulsion 57  
 ethylene oxide 517  
 eye drops 185, 515  
 for dry heat sterilisation 312  
 for infra red sterilisation 542  
 for radiation sterilisation 532  
 implants 546  
 inhalation 181  
 injection, see Injection container  
 instrument 543  
 insufflation 89  
 lotion 122  
 lozenge 97, 199  
 medical lubricant 314  
 metric 10  
 mixture 42  
 needle 433, 540  
 ointment 134  
 pastille 199  
 pill 95  
 plastic 350, 522, 523, 532  
 powder 78  
 snuff 89  
 spray solution 181  
 suppository 168  
 syringe 433, 539–40, 543  
 tablet 121  
 talc 315 519  
 throat paint 181  
 weighing 394, 443
- Contaminants  
 sources 400–2  
 types 400–2, 476
- Contamination  
 accidental 430, 452, 507  
 in sterility tests 343, 447, 448, 521  
 precautions against 450  
 reduction of 421–2  
 risk from  
 additional manipulations 454  
 air movements 402  
 ampoules 298, 361  
 aseptic distribution 435, 436  
 aseptic filling 447  
 aseptic sealing 400  
 assembly of distribution units 439  
 bacterial filtration 447  
 cooling of hot air oven 312  
 crevices in chairs 407  
 dressings containers 339, 340  
 drying of dressings 334  
 hand washing 405  
 handling of syringes 472  
 manipulations in sterility testing  
 469, 470
- Contamination, risk from—(contd.)  
 multi-dose containers 258, 294,  
 303, 306  
 multi-pack syringe containers 540  
 sterile ointments in jars 313  
 storage 312, 494  
 workers 402, 421
- Control of  
 aseptic processing 446, 447, 473  
 sterile processing 447, 473
- Controls, foot-operated 403, 405  
 sterility test, see Sterility tests
- Convection, see Heat transfer  
 Conveyor systems 530, 541, 542
- Cooling of  
 asepsis room air 410  
 culture media 546  
 instruments in air 543  
 multi-dose injections before sealing  
 385  
 transfusion bottles 337, 382
- Cord powder, sterilisation 315
- Corrosion  
 from aerial bactericides 416, 417  
 of metal injection units 298  
 of needles 538  
 of sintered metal filters 367
- Corticotropic zinc hydroxide, sterility test 463
- Cortisone acetate, crystal growth of  
 270  
 wettability 270
- Cortisone injections, bactericide in 259
- Cotton wool absorbent, for clarification of injections 366  
 contamination during manufacture 469
- Cotton wool  
 air filter 334, 369  
 coloured 313  
 ethylene oxide absorption 520  
 nitrogen filter 503  
 non-absorbent 413
- Cottonseed oil, injection vehicle 244
- Cover, aluminium foil, for mortar 507  
 powder ampoule 501
- Cracking of emulsions 75
- Cream 133, 136  
 bases, summary of 162  
 dispersal in sterility tests 450  
 face, for asepsis 409  
 hexachlorophane 424  
 of Magnesia 53  
 sterile, cooling of 405  
 sterilisation 546
- Creaming, of emulsions 71
- Cresol  
 absorption by rubber 293  
 bactericide in aqueous injections  
 259  
 bactericide in oily injections 260  
 in immunological preparations 261  
 inactivation in sterility tests 454,  
 459  
 incompatibilities 261, 557  
 pain on injection 261
- Cross-infection 402, 415, 529
- Cryoxide 517
- Crystal growth, in suspensions 270

## INDEX

**Crystallisation**  
 aseptic 479, 499, 517, 519, 522  
 calcium gluconate injection 393, 563  
 rubber particles as cause 394  
**Crystals**  
 protection of organisms by 519  
 sterilisation 519  
**Culture medium**  
 air space above 464  
 blood agar 233, 296, 474  
 Bonnel's 450  
 bouillon 226  
 Brewer's thioglycollate 229, 449  
 buffering capacity 457  
 caramelisation 546  
 casein hydrolysate 456  
 clarity 232, 453, 546  
 Clausen's 450  
 closures 229, 413, 434  
 confirmation of sterility 452, 458  
 constituents 226, 227, 234  
 containers 228, 229, 230, 232, 546  
 cooked meat 229, 230, 231, 232,  
     449, 458, 464  
 cooling before use 462, 498  
 copper in 226  
 corn steep thioglycollate 450  
 dehydrated 231, 233  
 desirable features 226  
 diagnostic 233  
 digest broth 226, 230, 231, 449  
 dilution of bactericides in 462  
 enriched 233  
 foaming during filtration 485  
 for aerobes 230, 449, 465  
 for anaerobes 229, 230, 449  
 for *Bacillus stearothermophilus* 344  
 for membrane filters 485  
 for moulds 234, 476  
 for sterility tests 230, 233, 343, 448–  
     50, 453, 459, 464, 465, 468, 470,  
     472 (see also Sterility tests)  
 formulation 452, 453, 458  
 glucose peptone broth 449  
 Hartley's digest broth, *see* digest  
 broth  
 honey broth 235  
 indicators, oxidation reduction, in  
     232, 449, 450  
 infusion broth 226  
*inhibition* in 450, 452, 472, 525  
 joint 446, 449, 450, 453, 459, 462,  
     470, 472  
 Iethene broth 456  
 Linden's thioglycollate 229, 449  
 liver broth 449, 450  
 MacConkey's bile salt 233  
 maintenance of sterility 410  
 malt agar 476  
 malt extract agar 476  
 malt extract broth 235  
 meat extract broth 226, 230  
 moisture content 226  
 nutrient agar 232, 418, 465, 466,  
     473, 474  
 nutrient broth 419, 442, 453, 519  
 nutrients in 226–227, 415, 546  
 oxidation reduction qualities 228,  
     452

**Culture medium—(contd.)**  
 oxygenation of anaerobic 232, 429,  
     462, 463, 464, 471  
 para amino benzoic acid (PABA)  
     broth 459, 467, 508  
 peptone broth 449  
 pH 227, 234, 457  
 Pittman's thioglycollate 449  
 preparation 226–233, 452, 453, 458  
 pretreatment of anaerobic 405, 449,  
     450, 452, 498  
 Robertson's 229  
 Sabouraud's, agar 476  
     fluid medium, saprophyte formula  
     235  
     fluid medium U.S.P. 235, 476  
     medium 234  
 sealing of 232  
 selective 233  
 semi fluid hydrosulphite 450  
 semi fluid meat 449  
 semi solid 232  
 sensitivity 225, 233, 447, 448, 449,  
     450, 453  
 sterilisation 230, 326, 347, 348, 452,  
     482, 546  
 storage 229–230 453  
 thioglycollate 229, 230, 232, 455,  
     458, 459, 462, 464, 465, 468, 471,  
     472, 498  
     broth medium U.S.P. 450  
     medium U.S.P., fluid 449, 450,  
     466, 471, 472  
     with cooked meat 232, 455, 464  
 tryptic digest 455  
 ultra violet action on 527  
 viscosity 449, 450  
 volumes for sterility tests 455, 462  
 Cupboard, dust proof 363, 373, 406, 498  
 Curare-like substances sterility test 446  
 Curie 530  
 Cyanocobalamin, bacterial growth fac-  
     tor 223  
 instability 265, 532  
 Cyanocobalamin Injection  
 instability 277  
 interference by bactericides in assay  
     261  
 sterility test 459  
 Cycle, sterilisation 343, 543  
 Cysteine  
 inactivator of streptomycin 457  
 protectant from radiation damage  
     533  
 L-cystine, in culture media 449  
 Cystoscopes, sterilisation 523

**D D T Application** 131  
 Dalton's law of partial pressures 320  
**Dangerous Drugs**  
 essential information on prescription  
     354, 386  
 retention of excess 374, 387  
**Death rate**  
 aerial bactericides 416, 417, 420  
 bactericides 454  
 ethylene oxide 522  
 ultra violet 420

**Decimal point** 355  
**Decomposition signs in injections** 277,  
     284  
**Dehumidification, asepsis room air** 410  
**Delayed release therapy** 117  
**Deliquescence, calcium chloride** 397  
**Deoxycortone Acetate**  
 implants 474  
 injection, solvents for 240, 244, 245  
 sterilisation 314  
**Depot therapy** 240 244, 249, 250, 267,  
     270, 271, 314, 474, 515, 545, 546  
     oral 117  
**Dermatitis from antibiotics** 298, 409  
**Desiccator:**  
 ampoule seal testing 372  
 ethylene oxide sterilisation 521  
**Detergents** 312, 362, 363, 407, 423, 430  
 anionic as antagonists for quats 456  
 cationic, precipitation in sterility tests  
     459  
 effects on rubber gloves 544  
 for syringe cleaning 537  
 non ionic, cleaning containers 373,  
     380, 382  
 with dye for ampoule seal testing  
     371  
**Dextran, pyrogen in** 364  
**Dextran Injection, survival of organ-**  
 isms in deposits 317  
**Dextrose, anhydrous** 381  
 adjustment to isotonicity with 254  
 caramelisation 381  
 culture medium ingredient 449  
 in hyperbaric injections 270  
 monohydrate 381  
 pyrogen in 364, 365  
 reducing agent in injections 268,  
     362  
**Dextrose Injection**  
 bacterial multiplication in 366  
 hypertonic solutions 459  
 isotonic solution 250, 253  
 labelling 357, 381  
 preparation 241, 337, 365, 380  
 pyrogen in 366  
 sterilisation 317  
 survival of organisms in deposits  
     317  
**Diagnostic preparations, sterility test**  
 446  
**Dichlorodifluoromethane** 517  
**Dick Test Toxin, isotonicity** 250  
**Dicophane, Application of** 131  
**Diffusers, ventilation systems** 421  
**Diffusible solids in mixtures, list of** 43  
**Diffusion**  
 bactericides into particles 416  
 ethylene oxide through plastics 522  
 free radicals 533  
 in air filters 411, 412  
**Digoxin Injection, vehicle** 245  
**Diluents, compressed tablets** 102  
**Dilute solutions** 21  
**Dilution coefficient** 454  
**Dimethylhydrate Injection,**  
 benzyl alcohol in 246, 349  
 propylene glycol solvent 349  
 sterilisation 349

- Dimercaprol, water solubility** 240  
**Dimercaprol Injection**  
 action on plastic syringes 287  
 ampoules obligatory for 314  
 as sequestering agent 268  
 benzyl benzoate in 245  
 labelling 358  
 sterilisation 314  
 sterility test 446  
**Dimethicone cream** 145  
**Diphenhydramine Injection, bactericides** in 261  
**Disc, cellulose film** 365, 378, 510  
**Discharge channel, steam steriliser**  
 324, 329, 330, 331, 332, 333, 336,  
 337, 343  
 air break in 332, 333  
 check valve in 332, 333  
 sight glass in 333  
 thermometer in 329  
 tun dish in 333  
 vapour escape line in 332, 333  
 water seal in 332, 333  
**Disinfectant fluids** 405, 407, 408,  
 517  
 detergent 407, 423, 456  
 for swabbing surfaces 422  
**Disinfection**  
 asepsis laboratory 408  
 humidifiers 410  
 skin 424-426  
**Disintegrants**  
 compressed tablets 102  
 implants 474  
**Disintegration**  
 compressed tablets 111, 112, 113  
 enteric coated tablets 111  
**Dispensing**  
 procedure 4  
 of proprietaries 215  
**Displacement values**  
 moulded tablets 86  
 suppositories 169  
**Disposable inhalers for medical gases**  
 220  
**Disposables** 298, 302  
**Distribution, aseptic** 301, 435-43, 479,  
 485, 499  
 ampoules with burette 436  
 ampoules with syringe 435  
 bottles with unit 438  
 large batches 438  
 units for 407, 436, 438, 439, 440,  
 485, 493  
**Domestic measures, equivalents** 566,  
 570  
 variation in 10  
**Domiphen bromide, as bactericide**  
 514  
 inactivation in sterility tests 456  
**Door**  
 asepsis laboratory 403, 406  
 hot air oven, opening of 312  
 mat 407  
 large steam steriliser 329  
 air separation at 324  
 condensation on 332  
 stepped, radiation sterilisation plant  
 533  
**Dose:**  
 accuracy of 270, 306, 393, 443  
 checking 354  
 number in multi-dose containers  
 294, 383  
 Schedule 4 poisons 354, 499  
**Draft Code on the Dispensing of Proprietaries** 215  
**Drain, see discharge channel**  
**Draught** 51  
**Dredger, powder** 507  
**Dressings**  
 air in interstices 323  
 aseptic handling 469  
 containers for sterilisation 339-41  
 B S box 339  
 cardboard box 340  
 fabric packs 331, 340, 345  
 metal drum 323, 329, 331, 332,  
 339, 343, 345  
 nylon film 286, 340  
 papers 340  
 deterioration in dry heat 314, 316  
 heat capacity 336  
 heat of absorption 321  
 heating up 316, 321, 331  
 known sterile 469  
 medicated 470  
 packing for sterilisation 319, 341  
 preheating 331  
 post-sterilisation drying 320, 321,  
 324, 334, 342  
 soaking of 320, 324  
 steam penetration 320, 329  
 sterilisation 316, 325, 328-35, 337,  
 339-42, 533  
 sterilisers for 318, 328-35  
 sterility testing 343, 469-71  
 storage 321  
 superheating 321  
**Droplet infection** 401, 409, 415  
**Drops** 50  
 ear 194  
 eye 184  
**Dry Granulation** 98  
**Dry gum method of emulsification** 56  
**Drying, aseptic** 517  
 dressings after sterilisation 334, 342  
 glassware after cleaning 362  
 after sterilisation 338, 439, 440  
 instruments 543  
**Dryness fraction, of steam** 324  
**Ducting, ventilation system** 410, 421,  
 528  
**Dummy packs, steam sterilisers** 343,  
 345  
**Dummy runs** 453  
**Dust**  
 attraction by plastics 285, 286  
 collectors 403, 404, 406, 410, 422,  
 423  
 control 405, 528  
 distribution 403, 410  
 hood 242, 392, 395, 437, 439, 489,  
 503  
 as powder funnel 507  
 in air 400, 401, 410, 416  
 micro-organisms in 401, 416  
 nucleus for crystal formation 393  
**Dust-(contd)**  
 protection 1  
 containers during drying 362  
 injections during preparation 378  
**Dust borne infection, simulation** 413,  
 420  
**Dusting, before injection preparation**  
 334  
**Dusting powder** 89, 314, 315, 479, 506  
 Absorbable B.P., ethylene oxide  
 sterilisation 522  
 packaging 507  
 penicillin and sulphathiazole 400,  
 506-9, 515  
 preparation of thermolabile 506-  
 509  
**Dyes**  
 adsorption by filters 482  
 for seal testing 379, 387  
 pyrogen test on intravenous 556  
**EAR**  
 cones 168  
 drops 194  
**E.D.T.A.** 268, 363, 514  
**Effervescent granules** 87  
**Efficiency**  
 air filters 412, 413, 414  
 bacterial filters 491, 493  
 bactericidal, ultra violet wavelengths  
 527  
 electrostatic precipitation 415  
 tests on air sterilisation techniques  
 418-21  
**Electrical diagnostic equipment, sterilisation**  
 517, 519  
**Electromagnetic waves** 526  
**Electrons, high velocity** 526, 527, 530,  
 531, 532  
 penetration 531, 532, 533  
 sterilisation by 484, 530, 532  
**Electron-volt (eV)** 527  
**Electrostatic attraction**  
 air filters 411, 412, 413, 414  
 liquid filters 493  
**Electrostatic charges, at low humidities**  
 410  
**Electrostatic precipitation** 411, 414-  
 15, 419, 420, 528  
 precipitator 414, 415  
**Elixir** 51  
**Emetine hydrochloride, cost** 266 374  
**Emetine injection, instability** 266, 276  
**Emulsification, oil sterility tests** 466  
**Emulsifier and mixer**  
 Kenwood 72  
 Q P 74  
 Silverson 73  
**Emulsifying agents:**  
 acacia 55, 56  
 agar 60  
 alginic acid 56, 63  
 anionic 56, 139, 143  
 beeswax 56, 66, 137, 138  
 cationic 56, 139, 144  
 cellulose derivatives 56, 62  
**Cetomacrogol Emulsifying Wax** 56,  
 141

**Emulsifying agents—(contd)**

- celostearyl alcohol 138
- Cetrimide Emulsifying Wax 56, 144
- choice of 68
- complementary 69
- Emulsifying Wax 56, 138
- glycerol monostearate 139
- glycerol esters 139
- glycol esters 139
- incompatibilities 213
- Lanette Wax SX 143, 166
- mucilage of Irish moss 55, 61
- non ionic 56, 139
- Polawax 142
- Polyethylene glycol derivatives 140
- esters of fatty acids 141
- ethers of fatty alcohols 141
- of sorbitan fatty acid esters 142
- saponins 56, 66
- self-emulsifying monostearin 139, 140
- soaps 56, 63–66
- sodium lauryl sulphate 138
- sorbitan esters 139, 140
- Spans 140
- starch 56, 66
- tragacanth 55, 61
- Tweens 140, 142
- wool alcohols 135, 136
- Emulsifying machinery 72, 73, 74
- Emulsifying Ointment 135, 138, 143, 160, 162
- Emulsifying Wax 56, 138, 143, 160
- Emulsion
  - concentration of disperse phase 59
  - containers 57
  - containing small quantities of disperse phase 59
  - cracking 75
  - creaming 71
  - distinguishing o/w and w/o 67
  - formulation 68
  - globule size 74
  - hexachlorophane 425
  - oil in water (o/w) 67, 139
  - phase inversion 75
  - phase volume ratio 70
  - preservatives 69
  - stability 70, 71, 75, 76
  - water in oil (w/o) 67, 139
- Energies, radiation 526, 527
- Enteric coating 95
- tests for 111
- Entrainment
  - in air filters 411
  - of organisms from masks 409
  - of pyrogen 241
- Envelopes
  - aluminum foil for catgut 467, 468
  - cellulose film for syringes 539
  - double paper for powders 315, 507
  - fabric for gloves 544
  - glassine for spore papers 344
  - heat-sealed, for distribution units 439
  - paper 440, 468, 508, 510, 522
    - as weighing containers 507
  - plastic 314, 417, 467, 522, 544
  - plastic-aluminum laminate 467

**Enzymes**

- adsorption by filters 482
- cellulase in moulds 484
- filtration sterilisation 492
- for cleaning filters 481
- types 456
- Epichlorhydrin, starch treatment for glove powder 544
- Equilibration solutions 293–4, 439
- Equipment
  - arrangement around screen 424
  - as contamination source 402
  - cleaning 318
  - sterilisation 312, 313, 316
- Equivalents, approximate in Metric & Imperial systems 13, 573
- Ergometrine Injection, instability 261, 268, 276, 366, 560
- Ergot alkaloids, instability 265, 268, 276
- Ergotamine tartrate, air replacement 268
- Ergotamine Tartrate Injection, instability 268, 276
- Errors
  - dispensing, sources of 4
  - displacement 383, 387
  - human, in steam sterilisation 337
  - weighing 394
- Erythema, from ultra violet 528
- Erythrocytes
  - agglutination 476
  - effect of paratonic solutions on 247, 248
  - fragility point 248
  - fragmentation in radiation sterilisation 532
  - haemolysis 247, 248
- Erythromycin, sterilisation 522
- Escherichia coli* 222, 223, 233, 463
- Essential metabolites 223, 455, 527, 546
- Esters, as injection vehicles 244, 313
- Ethanolamine Oleate Injection
  - bactericidal activity 259, 265, 317
  - precipitation in sterility tests 459
  - preparation 394
  - sclerosing agent 249
- Ethyl alcohol
  - catgut tubing fluid 467
  - cleaning ultra violet lamp 528
  - for swabbing 258, 422, 423, 432, 434, 437, 440, 441, 505
  - injection solvent 245
  - moulding agent, hypodermic tablets 545
  - opening ampoules with 431
  - skin disinfectant 424, 425–6
  - sterilisation of exterior of ampoules 430
- Ethyl cellulose 62, 151
- Ethyl gallate, as antioxidant 552
- Ethyl oleate, injection vehicle 244, 245
  - action on rubber 294
  - sterilisation 313
- Ethylene, medical gas 217
- Ethylene oxide
  - absorption 519, 520, 523
  - admixture with inert gases 517, 521

**Ethylene oxide—(contd)**

- airing after sterilisation 520
- antimicrobial activity 518
- chemical control 522
- compared with formaldehyde 524
- containers 517
- damage to plastics 523
- desorption 519, 520, 521, 523, 524
- factors affecting efficiency 518–20, 522, 523
- general properties 517
- humidity on 518, 519, 520, 522, 523
- inflammability 517, 524
- liquid, damage from 520, 523
- penetrating power 519, 524
- protection of organisms from 519
- reactivity 522, 524
- slowness 518, 523, 524
- sterilisation with 517, 518, 522, 524
- sterilisers 517, 518, 520–1
- sterility testing technique 450, 523
- toxicity 520, 524
- use in absence of oxygen 517
- vapourisation 520
- Evaluation, oil sterility tests 465
- Evaluation methods *see Tests*
- Evaporation of droplets in air 401, 416
- Excipients, pill 95
- Excitation 526, 527, 530
- Exposure, sterilising
  - automatic steam steriliser 332, 339, 342
  - closures 338
  - conducted heat steriliser 543
  - culture media 546
  - dressings and fabrics 325, 342
  - dry heat 309, 310, 312, 314, 315, 338, 343
  - ethylene oxide steriliser 518, 522, 524
  - fibrin foam 545
  - gas oven for syringes 542
  - glassware 338
  - infra red oven 345, 541
  - infra red vacuum oven 543
  - instruments 543
  - ionising radiations 531
  - manual steam steriliser 339
  - moist heat 316, 324, 343, 345
  - rubber gloves 544
  - saturated steam 324
  - steaming 317
  - Tyndallisation 348
  - ultra violet light 528
  - vaccines 346
- Eye discs, *see Lamellae*
- Eye drops 184
  - buffered 192
  - closure, rubber 185
    - absorption of preservative by 189
  - containers 185, 285, 287, 519, 523
  - formulation 184
  - hydrogen ion concentration 185
  - physiological activity and 263
  - isotonicity with lacrimal secretion 184, 191
  - preparation 185
  - preservatives 188, 189

**Eye drops—(contd)**  
 rapid adjustment to isotonicity 191  
 requirements 184  
 self-sterilising vehicles for 190  
 single-dose units 193  
 Solution for 189, 515  
 stability 184  
 steamer for preparation 186, 187  
 sterilisation 489, 490, 492  
 summary of B P C examples 193-4  
**Eye lotions** 125  
**Eye ointments** 134, 160  
 fibres in 511  
 preparation 400, 479, 509-11, 515  
 labelling 511  
**Eyes, protection from ultra violet** 409, 529

**FAN**  
 for distribution of aerial bactericides 417  
 for hot air oven 311, 312, 313  
**Fats, rancidity** 513, 532  
**Fermentation**  
 antibiotic, aeration 410, 411, 413, 420, 421  
 media sterilisation 347  
**Fermentation products**  
 pyrogen in 364, 555  
 reduction of yield 411  
**Fibres**  
 from filters 367, 369, 487  
 in eye ointments 313, 511  
 in injections 366, 371, 378  
**Fibrin foam** 545  
**Figures, alteration of** 355  
**Film, ampoule** 373, 375, 380, 422, 431  
**Filing, of ampoules** 375  
**Filling**  
 aseptic  
     liquids 436, 438, 447, 485, 529  
     ointments 314  
     powders 410, 499-503  
     devices 230, 438, 505, 506  
     injections 381, 394  
     under nitrogen 393  
**Film, cellulose, for protection in injection preparation** 378  
**Film, packaging**  
 cellulose 373, 380  
     amber 284, 385, 373  
 heat sealable 285  
 permeability 519  
 plastic 286, 519, 533

**Filter**  
 air 269, 301, 302, 339, 369, 392, 395, 411-14, 434, 439, 523  
 absolute 412, 414  
 asbestos 412, 414, 419  
 autoclave 334, 335, 338, 414  
 by-passing 413, 420  
 ceramic 335, 338  
 clogging 343, 410, 411, 413, 414  
 compaction 413, 414  
 Composite Kompak 412, 414  
 corrugated 412, 413, 414  
 cotton wool 334, 413, 414  
 efficiency 412-414

**Filter, air—(contd)**  
 fibre detachment from 413  
 fibrous 413  
 filter paper 412, 414  
 glass fibre 334, 335, 339, 412, 414  
 granules 412, 413  
 high efficiency 412  
 Kompak 412, 413, 414  
 laps 413, 414  
 merino wool 413, 419  
 methylene blue test 420  
 Multivex 413  
 packing density 413, 414  
 penetration by organisms 411, 413  
 prefilter 410, 414  
 resin treated 412, 413, 414  
 resistance to air flow 411, 413, 414  
 sampling 419  
 sodium flame test 420  
 testing 335, 419-20, 421, 476  
 types 412-14  
 wetting 413, 496  
 blood clot 301, 302, 303  
 bacteria proof 479-85  
 absorption by 488  
 adsorption, see Adsorption  
 air permeability 492  
 applications 490  
 asbestos pad 457, 474, 475, 479, 482, 495  
 Berkfeld 481, 490  
 bubble pressure 494  
 candle 479, 480, 481, 490, 492, 494  
 cellulose ester 479, 483, 484  
 cleaning 480, 481, 482, 483, 490, 493  
 clogging 480, 481, 482, 485, 489, 490, 491, 493  
 collodion membrane 483, 493  
 cost 482, 483, 484  
 desirable features 480  
 diatomaceous earth 479  
 disc 479, 492  
 disposable 479, 480, 482, 483, 490  
 Doulton Pasteur 480, 490  
 extraction from 480, 482, 483, 484, 490, 491, 493  
 fibres from 482, 487  
 fibrous pad 479, 482, 490, 493  
 Gradocel membrane 483, 484, 493  
 Hemmungs 487, 488, 489  
 kieselguhr 481, 482, 492, 493  
 Mandier 481, 492  
 manufacture 479, 480, 483  
 maximum pore size 480, 490, 491, 492  
 membrane 457, 475, 484, 489, 490, 491, 492, 493  
 cultivation of bacteria on 484, 485  
 Membranfiltrern 484  
 microporous plastic 479, 483, 488, 493

**Filter, bacteria-proof—(contd)**  
 Millipore 484  
 mountings 480, 481  
 Oxoid 484  
 particles from 484, 493  
 Pasteur-Chamberland 480  
 penetration by bacteria 483, 488, 491, 493  
 porosity 480, 481, 483, 484  
 pressure resistance 482, 483  
 retention of liquid in 479, 493, 494  
 Seitz 482  
 Selas 480  
 sintered ceramics 479, 480, 490, 491, 493  
 sintered glass 5 on 3 479, 483, 489, 490, 491, 493, 495, 497  
 sterilisation 480, 484, 489, 490, 493  
 Sterimat 482, 488, 490, 493  
 storage 481, 483, 484, 493  
 strength 480, 481, 482, 483, 493  
 supports for 487, 488, 490  
 tests 490-2, 493  
 unglazed porcelain 479, 480, 481, 492, 493  
 unmounted 480  
 clarifying 366, 480, 487, 491  
 adsorption of medicament 366  
 cellulose ester 367, 368  
 glass fibre 485  
 jacketed 390, 393  
 manufacture 483  
 nylon 487  
 particles from 366  
 plastic 367  
 prewashing 366  
 sintered 367, 487  
     glass 366, 367, 369, 378, 381, 385, 390, 393, 395, 479, 480, 482, 515  
**holder, bacteria proof filters** 475, 480  
 Buchner 483, 488, 496  
 centrifuge 484, 487-8  
 fibrous pad 482, 486, 487  
 glass 487, 489  
 Gradocel membrane 483  
 large fibrous pad 487  
 membrane 489  
 metal 485, 486, 487  
 pipe line 483, 485  
 syringe 484, 489  
**holder, clarifying filters** 366, 367, 369  
 paper  
     cellulose asbestos 412, 414  
     glass 412, 414  
     press 487, 490  
 pyrogen retaining 365  
**Filtration**  
 air 334, 410, 411-4, 415  
 for clarification  
     eye ointments 510  
     gravity 366, 378, 385, 389, 390  
     oils 390  
     vacuum 366, 381, 385, 389, 390, 393, 396

## INDEX

- Filtration—(contd)  
for sterilisation.  
alcoholic solutions 482, 494  
alternative to heat 490  
eye drops 515  
mechanisms 480, 483, 493-4  
oils 475, 482, 490  
pressure 480, 482, 485, 491, 493  
technique 481, 482  
vacuum 480, 482, 485, 493, 496  
viruses 483  
viscous preparations 482, 546
- Filtration unit  
bacteria proof filter 480, 485-90,  
493, 495  
Barfield 498  
batteries of candles 485  
centrifuge 487, 488, 489  
cleaning 488  
Clinbrine 485  
fibrous pad 485, 486, 487, 488, 493  
joints 485, 493  
leaks 446, 483, 491, 493  
membrane 488, 489, 490  
pressure 485, 486  
sintered ceramic 481, 485, 486  
sintered glass 488, 490  
sterilisation 489-90  
syringe 489  
vacuum 485, 486  
clarifying filter 368, 369, 385
- Flaking, *see* Glass
- Flaming 309, 468  
aseptic technique 424, 427, 430,  
437, 440  
glass boat 443, 502  
metal scoop 502  
tile 422, 424  
tubes 427, 429, 431
- Flavour, in emulsions 70
- Flooring, asepsis laboratory 403-4,  
407
- Flow properties of powders 314, 315,  
544
- Flow rate  
air in filters 413  
air in samplers 418, 419  
air over ultra-violet tubes 528  
bacteria proof filters 475, 480, 481,  
482, 483, 484, 490, 492  
carbon dioxide 395  
clarifying filters 367, 368, 385  
nitrogen 392, 503
- Flowmeters, gas cylinders 219
- Foaming  
in pasteurisation 347  
in siliconised containers 280  
of filtrates 485  
of suspensions 270
- Footwear, for asepsis 409
- Forceps, use in asepsis 313, 422, 426,  
427, 431, 432, 434, 468
- Formaldehyde 348, 517  
compared with ethylene oxide 524  
inactivation by organic matter 524  
penetrating power 524  
polymers 524  
preparation of glove powder 544  
Solution 524
- Formaldehyde—(contd)  
sterilisation with gaseous 523, 524,  
545  
sterilisation with liquid 468  
toxicity 524  
vapourisation 524
- Formalin 524
- Formulac, injection 354, 355, 490
- Formulation of  
emulsions 68  
eye drops 184  
injections 237-71  
osmotic pressure 246-58  
pH 265-7  
preservatives 258-61  
specific gravity 269-70  
suspensions 270-1  
vehicle 240-6  
volume 239, 296  
pills 93  
tablets 102
- Fractional quantities, of a formula 17  
to obtain 39
- Fragility of blood cells 247, 248
- Free radicals  
in autoxidation 550  
in radiation sterilisation 531, 533
- Freeze-drying  
fibra foam 545  
gelatin sponge 545  
penicillinase 457
- Freezing points, table of 250
- Freezing point depression 250, 255
- Fumigation, formaldehyde 524
- Fungi  
destruction of filter 484  
pathogenic 234
- Fungicide 235
- Fungistat 235, 484
- Funnel  
jacketed for eye ointments 510  
powder 302, 507
- Furniture, asepsis laboratory 406-7,  
408
- Gall and Opium Ointment 147
- Gallic acid 551, 552  
esters 551, 552
- Gallipot 422, 423, 505
- Gamma rays 526, 529, 530, 531  
sterilisation techniques 531, 532
- Gas  
inert 298, 392, 517  
medical 217  
supply, asepsis laboratory 405
- Gas gangrene 314, 467
- Gauze 339, 469, 470, 545
- Geiger Muller counter, sterilisation  
523
- Gelatin  
capsules 196  
flexible 196  
hard 84  
manufacture of 197  
rotary machine 197  
seamless 197  
soft 196  
in culture media 348, 471
- Gelatin—(contd)  
glove powder 544  
pastes 149  
sponge 316, 545
- Gelato-glycerin 167
- Gelling agents 271, 314
- Giving sets 286, 287, 301, 302, 303,  
523
- Glass  
alkali from, *see* Alkalinity of glass  
alkalinity tests, *see* Tests  
annealing of 369, 376  
borosilicate 279, 306, 479, 483, 487,  
527, 537, 539  
chemical durability 274, 278, 279  
cleaning 354, 361-3  
colourless 284, 304  
composition 274-5, 278-9, 283, 379  
cost 274, 278, 279  
degreasing 312, 338, 362, 423  
extinction coefficient 283, 284  
flaking 274, 278, 279, 280, 300, 359  
for injection solids 277  
for light protection 283-4, 297, 304  
light transmission 283, 284  
lime soda 275, 278-9, 297  
loss of brilliance 277-8, 279, 338,  
362  
manufacture 274, 279  
mechanical strength 278, 300  
medicine bottle 275, 278  
melting of 279, 297, 379  
neutral 229, 279, 297, 306, 537  
neutral tubing for ampoules 279,  
297, 379  
non-active 283, 284  
quartz 527  
radiation sensitivity 532  
silica 274  
silicone-treated 279-280  
spicules 297, 298  
splintering 297, 376  
structure 274-5  
sulphured 278, 279, 281, 306  
surface treated, *see* Glass, sulphured  
tests 278, 280-3  
thermal resistance 274, 278, 279,  
297, 300, 336, 337  
thickness 278, 284  
ultra violet absorbing impurities  
527
- U.S.P types 282  
visibility of contents 284
- Vycor 527
- weathering 277, 279, 281
- Glassware, sterilisation 312, 313, 316,  
319, 338
- Globia Zinc Insulin Injection 277
- Glove powders 544  
Absorbable B.P. 544  
sterilisation 316, 520, 544
- Gloves, rubber 408, 409, 470, 513,  
529, 544  
manufacture 289, 290  
packing for sterilisation 344  
sterilisation 325, 332, 337, 409, 519,  
520, 523, 544
- Glucose, as reducing agent 550
- Glycerin, sterilisation 314

- Glycerin of  
Borax 183  
Ichthammol 183  
Phenol 183  
Starch 150, 183  
Tannic Acid 183  
Glycerin soap suppositories 178  
Glycerin suppositories 177  
bases 167  
Glycitol esters as emulsifying agents 139  
as suppository bases 167  
Glyco-gelatine, for lamellae 545  
Glycol esters, as emulsifying agents 139  
Glycols, as aerial bactericides 417  
Gowns 408, 422, 423, 529  
Granulation  
by Preliminary Compression 98, 101  
Dry 98  
Moist 98, 99  
Granules  
effervescent 87  
for compressed tablets 98  
Grease, removal, from instruments 543  
Gregory's Powder 92  
Growth factors 223, 227, 344, 455, 459, 496
- HAEMOLYSIS 247, 255, 296, 466  
Haemostatics, absorbable 316, 446, 545  
Hair, contamination source 402, 408  
Halden's emulsifying base 143  
Half life 530  
Hands  
contamination source 401  
disinfection 422, 424-6  
preparation for asepsis 403, 405, 409, 424-6, 495  
Hand-driers 405, 426  
Handling, aseptic 499, 519, 533  
Hard gelatin capsules 84  
Hartmann's solution 396  
Head-dress, aseptic 408, 409, 529  
Heat, sensible 319  
Heat of absorption, dressings 321  
Heat transfer 312, 315, 319, 320, 323, 350, 541  
conduction 309, 311, 322, 324, 336, 337, 541, 542  
convection 309, 311, 313, 541, 542, 542  
radiation 309, 311, 312, 314, 541  
Heating, air for asepsis laboratory 410  
Heating in an Autoclave, 316-46, 396, 400, 476, 484  
advantages and disadvantages 342  
B P method 324  
culture media 546  
equilibration of closures with bactericides 293  
filtration units 489, 496  
injections sterilised by 373, 380, 382, 558  
jellies in tubes 546  
membrane filters 484  
M.R.C. recommendations 324  
packaging for 319
- Heating in an Autoclave—(contd)  
post sterilisation drying 496  
thermostable plastics 285, 286, 287, 303, 489  
syringes 536, 540, 541  
rubber closures 291, 295  
tests for alkalinity 281, 282  
Heating up  
autoclaving 345, 546  
conveyor oven 541  
Heating with a Bactericide 387  
high vacuum steriliser 331  
hot air oven 311, 313  
infra red vacuum oven 543  
instrument loads 543  
surgical dressings steriliser 336, 342  
syringe tubes 40  
times 343, 379, 507  
Heating with a Bactericide 239, 259, 276, 317, 348, 50, 383, 394, 400  
apparatus for 349, 350  
injections sterilised by 385, 388, 553-9  
labelling of injections 359-61  
plastic containers 285  
sterility test on injections 459  
theory of 224  
H E B 143  
Helium, medical gas 217  
Heparin Injection  
adsorption by filters 365  
instability 277, 366, 532  
labelling 498, 499  
loss of bactericide from 294  
pyrogen in 364, 365  
sterility test 446  
Hexachlorophane 424-6  
Hexamethonium, phenol incompatibility 557  
Hexobarbitone sodium, sterility test 454  
Hexylresorcinol, aerial bactericide 417  
Histamine Acid Phosphate Injection 365, 373-80  
H.L.B. 70  
Holding, aseptic technique  
closures 427, 429, 435  
containers 431, 435, 473  
Pasteur pipette 428  
syringe 434  
teat 423  
tubes 427, 428, 463  
Homogenisers 74  
Homologous serum jaundice, heat resistance 316  
Hot air oven, see Oven  
Hour-glass devices 346  
Humidity  
aerial bactericides and 416, 418, 420  
asepsis laboratory 404, 410  
chemical sterilisation indicators and 346  
effect on  
droplet evaporation 401  
dust sedimentation 401  
filter papers for air 414  
gaseous sterilisation 518, 520, 521, 522, 524  
ultra violet air sterilisation 528
- Hyaluronidase 239, 364, 446, 492  
Hydrocortisone acetate:  
test for sterility 463  
wettability 270  
Hydrocortisone injection, vehicle 245  
Hydrogen ion concentration, see pH  
Hydrogen Peroxide  
from ionising radiations 531  
from ultra-violet on culture media 527  
Hydrogenated oils, as suppository bases 167  
Hydrophilic-Lipophile Balance (H.L.B.) 70  
Hydrophilic petrolatum 135  
Hydrous Emulsifying Ointment (Aqueous Cream) 139, 143  
Hydrous Ointment (Oily Cream) 133, 134, 137  
Hydrous Wool Fat 136  
para-Hydroxybenzoic acid esters  
antifungal activity 235, 484  
binding by nylon 286  
inactivation in sterility tests 454, 459  
Hydroxylaniline, streptomycin inactivator 457  
Hygroscopic substances  
in compressed tablets 99  
in powders 78  
Hygroscopicity, penicillin 410  
Hyoscine Eye Ointment 509  
Injection 276, 360  
Hypochlorites  
aerial bactericides 417  
for filter cleaning 481, 482  
Hypodermic solution tablets 105, 240, 345  
Hypophosphorous acid, reducing agent 549, 550
- IMHAUSER (Witepsol) suppository bases 167  
Immunological preparations  
bactericides for 259, 261  
sterilisation 481, 490, 492  
sterility tests 446, 472  
Imperial system 9, 11  
Impingement  
in air filtration 411, 412, 414  
in air samplers 418, 419  
of spray in stills 241  
Implants 97, 446, 474, 545, 546  
Inaccuracies, in dispensing 5  
Inactivators, in sterility tests 454-7, 475  
Incompatibility 202  
adjusted 202, 208  
alkaloids with  
alkalis 203  
benzoates 206  
bromides 206  
iodides 206  
salicylates 206  
tannic acid 206  
ammonium bicarbonate with acid  
squill preparations 210  
bactericides in injections 261, 382

## INDEX

- Incompatibility—(contd.)  
 barbiturates, soluble, with.  
 acids 212  
 ammonium bromide 211  
 benzoates and salicylates with.  
 acids 208  
 ferric salts 209  
 bicarbonates with  
 calcium salts 210  
 magnesium salts 210  
 bismuth salicylate with sodium bicarbonate 210  
 bismuth subnitrate with sodium bicarbonate 210  
 chemical 202  
 emulsifying agents 213  
 iodides with ferric salts 207  
 iron salts with tannins 211  
 liquorice extract 211  
 physical 202  
 salicylates, see benzoates  
 sodium bicarbonate with  
 bismuth salts 210  
 borax and glycerin 210  
 tolerated 202, 208  
 unintentional 202
- Incubation conditions  
 bacterial spore papers 344  
 filter efficiency test 491  
 membrane filters 484, 485  
 penicillinase production 456, 457  
 penicillinase with penicillin 509  
 plate cultures 474  
 sterility test  
 bacterial 223, 343, 450, 451,  
 467  
 catgut 467  
 mould 476  
 viral 476  
 subcultures 459
- Indicator  
 chemical, of sterilising exposure  
 344, 345, 347, 522, 532  
 oxidation-reduction 232, 449, 450  
 paper 266, 366
- Indissoluble solids in mixtures 45, 46
- Inflammability  
 air filters 414  
 ethylene oxide 517  
 solvents for syringe lubricants 539
- Infra red sterilisation, *see* Sterilisation
- Infusion, concentrated 43
- Infusion fluid 237, 396, 397  
 administration 301, 303  
 bactericides in 260, 300, 364, 380  
 containers 279, 282, 286, 296, 299  
 flakes in 278  
 isotonicity 239  
 labelling 260, 357, 380  
 milliequivalent calculations 263  
 particles in 381, 382  
 preparation 380-2, 402  
 pyrogen in 240, 241, 364, 555  
 sterilisation 318, 322, 382  
 sterility test 432, 462
- Inhalation 181
- Inhaler, medical gas 220
- Inhibitors, in sterility tests 452, 463  
 of viruses 476
- Injection  
 adjuncts 350, 393, 515  
 bactericides 258-61, 294, 317, 389  
 buffers 265, 515  
 calculations 355  
 clarification 368, 404  
 closures 288-96, 301, 385  
 containers 274-307, 314, 391 (*see also* Injection containers)  
 cooling 278  
 decomposition signs 277  
 depot 249  
 diagnostic 237, 249, 250, 472, 543  
 emulsion 240  
 formulation, *see* Formulation  
 from sterile solid 479, 505-6  
 hyperbaric 270  
 hypertonic 249, 250, 459  
 hypobaric 270  
 hypodermic 237  
 intracisternal 239, 260, 350  
 intracutaneous 237, 238, 239, 249,  
 250  
 intramuscular 237, 238, 239, 246,  
 249, 250, 349, 474  
 intrathecal 238, 239, 249, 260, 350,  
 371, 389, 515  
 intravenous 237, 238, 245, 265, 301,  
 394, 395, 396, 397, 555, 563  
 bactericides in 239, 260, 389  
 flakes in 278  
 infusion fluids, *see* Infusion fluids  
 isotonicity 239, 247, 248, 249, 250  
 pyrogen in 241, 364  
 risk from oils 240  
 slow administration 245, 246,  
 249, 265  
 volume 239, 248, 250
- isobane 270
- isotonicity 239, 247-50
- issue 447, 479, 498
- mobile 374
- multi-dose  
 bactericides in 258-9, 294, 317  
 labelling 358, 360, 498  
 preparation 303, 378, 382-6,  
 388-9, 391, 457  
 sterility test 349, 494, 391  
 sterility test 432, 453, 459, 460, 462
- oily 237, 240, 249, 546  
 bactericides in 260  
 clarification 390, 482  
 closures 294, 391  
 containers 382, 314  
 labelling 240, 244, 356, 357, 359,  
 360, 361, 390  
 overage 374, 389  
 plastic syringe for 299  
 preparation 389-91  
 sterilisation 310, 312, 314, 350,  
 391, 490  
 suspensions, *see* Injections, sus-  
 pensions, oily  
 vehicles 244
- needles 537
- overage 374, 389
- overseals 372
- oxygen sensitive 292
- pain 246, 265, 276, 349
- Injection—(contd.)  
 particles in 371  
 peridural 238, 239, 260, 350  
 pH 265-7, 276  
 preparation 354-97, 479-506  
 aseptic 464, 503-6  
 examples 373-97  
 filling 370  
 filtration 366-9  
 immediately before use 270, 400,  
 545  
 in syringe 545  
 inspection 370-371  
 multi-dose 382-6, 388-9, 391  
 nitrogen filling 392-3, 503  
 packaging 354, 373  
 pH adjustment 266, 394-5  
 polishing 354  
 protection during 241, 318, 365  
 saturation with carbon dioxide  
 395, 396  
 sealing 370, 372  
 single dose, large volume 380-2  
 single dose, small volume 373-80,  
 389-91  
 soap solution 394  
 supersaturated solution 393-4  
 self sterilising 317  
 single-dose, large volume 296, 299,  
 337, 358, 380-2, 459, 563  
 single-dose, small volume 373-80,  
 385, 389, 459, 494  
 stabilisation 267-9, 354, 560-3  
 sterilisation methods 558-9  
 sterility tests 446, 447, 453, 459  
 storage 278  
 subcutaneous 237, 238, 239, 246,  
 249, 250, 265, 474  
 suspending agents 393  
 suspensions, aqueous 237, 249, 393,  
 515  
 container 280  
 preparation 393  
 sterilisation 338  
 sterility test 446  
 suspensions, oily 237, 240, 249, 350,  
 515  
 aseptic preparation 314  
 sterilisation 314  
 sterility test 446  
 thermolabile 400  
 thermolabile 371, 400  
 types 240, 354  
 viscous 374  
 volume 239, 248, 249, 250, 293
- Injection container 274-307
- antibiotic vial 279, 299, 305, 306
- B P requirements 296
- Buffered Solution Tablets of Peni-  
 cillin 515
- bursting of 312
- cartridge, *see* Cartridge
- chipped 363, 381
- cleaning 274, 299, 317, 318, 361-3
- Clinbrite 304-5, 383, 439
- Clinbrite Mark I 304, 305, 432
- Clinbrite Mark II 305, 306, 432
- colour 274, 284
- cost 274, 299, 306

- Injection container—(contd.)  
 damage by moist heat 316  
 desirable features 274  
 drying 278  
 effect on therapeutic properties of contents 296  
 for ethylene oxide sterilisation 450, 522, 524  
 for intravenous fluids 301  
 for protection from light 283–4  
 for radiation sterilisation 532  
 for solids for injection 499  
 gas tight 269, 389  
 glass, sterilisation 312, 329, 338, 350  
 McCartney bottle 383, 391, 397, 437  
 for solvents 505  
 for sterile fluids 301  
 mechanical strength 278, 299, 300, 306  
 minimum size 391  
 multi-dose 303, 395  
 aseptic handling 432  
 for oily injections 314, 391  
 for powders 305, 306, 499  
 for suspensions 393  
 for Water for Injection 499  
 loss of bactericides from 293  
 loss of solvent from 245, 306, 505  
 number of doses in 294, 307  
 sealing 370  
 storage 307  
 unsuitability for supersaturated solutions 393  
 waste with 306  
 withdrawal of doses from 258  
 particles from 296  
 post-sterilisation cooling 278, 382  
 reaction with medicaments 296  
 repeated use 274, 278, 306  
 rubber-capped vial 281, 282, 306, 519, 520  
 sealing to exclude micro-organisms 296  
 silicone treated, see Glass, silicone-treated  
 single dose & multi-dose 306  
 storage 278, 363  
 sulphured, see Glass, sulphured  
 surface treated, see Glass, sulphured  
 tests 306  
 thermal resistance 300  
 transfusion bottle, B.S. 269, 296, 300, 301, 369, 373, 383, 396  
 aseptic handling 433, 434  
 filling 370, 381  
 inspection unit 371  
 sealing 370  
 sterility testing technique 432  
 types 296–307  
 units 298–299  
 automatic injector 298, 299  
 plastic syringe 299  
 Tubunc syringe 298  
 use of w.c. 362, 363, 385  
 visibility of contents 274, 277, 287, 371  
 weight 306  
 well-closed 392  
 well-filled 392
- Inoculum size 225, 448, 449, 451, 453, 454, 459, 460, 476  
 Inspection  
 cleaned containers 363  
 injections for particles 370–1, 385  
 unit 371  
 Instruments  
 cleaning 543  
 packing for sterilisation 286, 319  
 protection of cutting edges 543  
 sterilisation  
 beta propiolactone 524  
 dry heat 313  
 infra red in high vacuum 543  
 steam 332, 337, 543
- Insulin Injection  
 depot forms 267  
 instability 265, 276, 292, 482, 532  
 mould growth in 234, 265  
 pH 265, 277  
 phenol as bactericide 265  
 sterilisation 490, 492  
 sterility test 446  
 syringes for 537
- Insulin Zinc Suspensions, acetate buffer in 267  
 crystal shape and size in 270  
 depot effect 267
- Integrator, West's time temperature 337, 346
- Interception, in air filtration 411, 412
- Iodine  
 action on stainless steel 404  
 in powders 90  
 inactivation in sterility tests 467, 468  
 ointment 147  
 non-staining 148  
 sterilisation of catgut 467–8  
 swabbing with 258, 468, 470
- Iodised Oil Injections 240, 265
- Iodophors, asepsis laboratory disinfectants 408
- Ion exchange resins, pyrogen removal by 243
- Ionisation 526, 530, 532, 415
- Irish moss emulsions 55, 61
- Iron impurity  
 in glass 284, 527  
 in water 528
- Isoosmotic solutions 247
- Isoprenaline spray 549  
 compound 549
- Isopropyl alcohol  
 catgut tubing fluid 467  
 sterilisation foil packs 468  
 swabbing with 505
- Isopropyl myristate, injection vehicle 245
- Isononic solutions 247  
 adjusting substances 250, 258  
 calculation methods  
 B.P.C. 250  
 freezing point 250  
 International Pharmacopoeia 258  
 molecular concentration 253  
 molecular weight 255  
 definition 247  
 milliequivalents in 264
- Isotonicity 248, 250, 393, 396  
 adjustment to 248, 250–8  
 injections 247–50  
 ophthalmic solutions 125, 184, 191, 250  
 topical preparations 250
- JACKET, ethylene oxide steriliser 520  
 Jacket, steam steriliser 329, 331, 332, 333, 335, 336, 337, 342  
 drying dressings with 324, 334  
 preheating dressings by 321
- Jacket, ultra-violet lamp 528
- Jar  
 for sterile powders 464, 501  
 for sterility tests 465, 466, 468, 470, 471  
 ointment for sterile products 313, 314  
 swab 422, 423, 424
- Jellies 133, 149
- sterilisation in tubes 546
- KAOLIN 314, 480  
 mixtures 44  
 poultice 162
- Kenwood mixer 72
- Keratin coating, pills and capsules 95  
 testing of 111
- Kupp's measure 230, 474
- LABELLING of injections 354, 356–61  
 498–9, 500–1  
 accuracy 356  
 adjuncts 357  
 ampoule 259, 356, 357–60, 380, 498, 500  
 ampoule box 357–8, 359–60, 498–9, 500–1  
 bactericide concentration 357–61, 498–9  
 batch number 498–9, 515  
 B.P. or B.P.C. requirements 356, 357  
 cleanliness 356  
 danger from crystal separation 394  
 danger from glass spicules 357, 359  
 date of manufacture 356–61, 498–9  
 date of sterilisation 357–61  
 D.D.A. requirements 356–8, 360–1  
 dilution instructions 357  
 exercises in 397  
 expiry date 294, 498–9, 515  
 identification after autoclaving 379  
 infusion fluids 260 357–9, 380  
 licence number 498–9, 515  
 methods 361  
 milliequivalents 357  
 name and address of manufacturer 498–9  
 name of preparation 357–61, 498–9, 500–1  
 oily injections 240, 244, 357  
 Pharmacy & Poisons Act requirements 356–61, 384  
 protection from light 357, 359, 360, 384, 385, 392

## INDEX

- Labelling of injections—(contd.)  
 reference number 357-61  
 route for injection 357, 499  
 sclerosing agents 394  
 solids for injection 500-1  
 solvent 357, 499  
 special instructions 354, 356  
 storage 498, 505  
 strength 357-61, 384, 498-9  
 suspensions 393, 515  
 transfusion bottle 300, 382  
 T.S.A. requirements 356, 358,  
 498-9  
 siliconed vials 280  
 volume 357, 359-61
- Labels 4, 7  
 checking 365  
 detachment 361  
 dressing packs 342  
 eye ointments 510, 511  
 fixing to ampoules 357  
 flag labelling 356  
 for plastics 285, 286  
 in asepsis 423  
 peeling from tubes 510  
 moistening 423  
 petri dishes 473  
 radiation sensitive paint for 532  
 specimen, for injections 358-61,  
 488-9, 500-1  
 sterile dusting powders 506-7  
 sterility test containers 458, 459,  
 460, 462  
 types 361, 422, 423, 462, 510  
 writing of 354, 361, 423
- Laboratory  
 asepsis  
   cleaning 407  
   design 402-7  
   ventilation 409-21  
 bacteriological, for sterility test controls 453, 463  
 note-book 3  
 rules 3
- Labraflils, injection vehicles 245  
 Lachrymal secretion, osmotic pressure 184, 191  
 Lactose, sterilisation 315  
 Lag, bacterial growth phase 225  
 time in sterilisation processes 311  
 312, 319, 382, 459, 510  
 Heating with a Bactericide 350  
 hot air oven 311, 312, 313, 314,  
 338, 391  
 steam sterilisation 326, 331  
 Lagging 310, 329, 332, 335, 493  
 Lamellae 545  
 Lamps ultra violet 527-8, 529  
 Lanette Wax S X 143  
 Lanolin 136, 159  
 anhydrous 136, 159  
 Laps 413, 414  
 Lard 133, 159  
 benzoinated 156, 159  
 Lassar's Paste 133, 162  
 Latent heat 319, 321  
 Latin terms 568  
 Lecithin, antagonist for quats 456  
 Leptazol Injection 259, 277, 304, 317
- Ligatures 446, 467-9  
 Light  
   action on bacteria 224  
   protection from 265, 276, 283, 357,  
 373, 392, 393, 494  
   by box or paper 284, 297  
   by plastics 287  
   injections requiring 560  
   of rubber 289, 305  
   transmission of glass 284  
 Light petroleum, in sterility tests 366,  
 475  
 Lighting asepsis laboratory 403, 404,  
 405  
 Lidocaine and Adrenalin Injection,  
 reducing agent in 268, 549  
 Lime creams 138  
 Linctuses 51  
 Liner  
   card 314, 443  
   Clenbuterol bottle 304, 385  
   rubber 228, 314, 338, 382, 443  
 Liniment 122, 129, 130  
 Aconite 129  
 Aconite, Belladonna and Chloroform 129  
 Belladonna 129  
 BP and BPC examples 129  
 Camphor (Camphorated Oil) 129  
 Ammoniated 129  
 Methyl Salicylate 130  
 Soap 18, 130  
 Turpentine 130  
 Liquid paraffin  
   sterilisation 313, 314, 471  
   syringe lubricant 538, 541  
   use in sterility tests 466, 471  
 Loading  
   ethylene oxide screen 523  
   hot air oven 311, 312, 314, 391  
   steam steriliser 323 326, 330, 331,  
 335 341-2 469  
 Lobeline Injection 261  
 Lotion 122  
   Calamine 127  
   Calamine Oily 17, 18, 130  
   Copper and Zinc Sulphate 130  
   eye 125  
   Hydrocortisone 130  
   insoluble substances in 127  
   Lead 122  
   Lead, Evaporating 130  
   precipitated sulphur in 128  
   Salicylic Acid and Mercuric Chloride  
 130  
   simple 122  
   Zinc Sulphate 130  
 Lozenges BPC 200 201  
 made by compression 201  
 made by traditional method 199,  
 200  
 Lubricant  
   compressed tablet 101  
   glove 544  
   medical 313  
   stopcock 436  
   suppository 170  
   syringe 316 538-9, 541  
 Lysol 463, 468, 474, 543
- McCARTNEY bottles, see Injection containers  
 Machine  
   foil capping 540  
   syringe cleaning 538  
 Macrogol Ointment 161  
   pastes 161  
   suppository bases 167  
 Magnesium carbonate, mixtures 44  
 Magnesium Hydroxide Mixture 55  
 Mandi's Paint 183  
 Manipulation losses 389, 509  
 Masks 409, 529  
 Massa esterum (suppository base) 167  
 Massupol, suppository bases 167  
 Measuring, errors in 5  
 Median basilic vein 238, 249  
 Medical gases 217  
   cylinder care 218  
   fine adjustment valves 219  
   flowmeters 219  
   humidifiers 220  
   inhalers 220  
   regulators 219  
   safety precautions 217  
   uses 217  
 Medicament  
   adsorption, by filters 365, 366, 368,  
 457, 481, 482, 483, 493  
   during pyrogen removal 364, 365  
   antibacterial 317, 453, 467, 474,  
 475  
   in sterility tests 453-8, 459, 464,  
 467, 474, 475  
   apyrogenic 365  
   oxidisable 362, 505  
   quality 364-5  
   stability, in injections 265-9, 314,  
 354, 392  
   in solution 400, 479, 493, 499  
   to dry heat sterilisation 310, 313  
   thermolabile 400, 446, 479, 529  
   sterilisation 317, 350, 492, 517,  
 523, 528, 529  
 Melarsoprol Injection, propylene glycol solvent 246  
 Meninges 238, 260  
 Mephenesin Injection, protection from alkali 277  
   propylene glycol solvent 246  
 Mercurials, adsorption on bacteria 475  
   inactivation in sterility tests 435  
 Mercuric iodide lotion 123  
 Mersalyl Injection, chlorocresol in-compatibility 350  
   preparation 269, 355, 394-5  
   stability 269, 277  
 Mesophiles 223, 243  
 Metaffiltration, for injections 368  
 Metal  
   contact avoidance 392, 393, 480,  
 485, 487, 489, 513, 562, 563  
   harmful effects of traces 267, 268,  
 289, 292  
 Methicillin sodium, sterility test 475  
 Methylcellulose  
   emulsions 56, 62  
   in eye drops 184

- Methylene blue  
oxidation-reduction indicator 232  
penetration of air filters 414  
test for air filters 412, 420
- Methylergometrine maleate, air replacement for 268
- Methylergometrine injection, glass ampoules on 276
- Methyldihydroxy benzoate in emulsions 193  
eye drops 193
- Metric system 9, 14
- MeV 527
- Micro-organisms  
death rate 318  
for sterility test controls 457, 460, 463  
in air 400-1, 528  
in cotton wool 334  
in dust 400-1, 528  
oil-coated in sterility tests 465
- Milk, pasteurisation 347
- Millimol 264
- Millieosmol 264
- Milliequivalents 261-5  
calculations 263  
labelling 358, 359, 397
- Mills, sterilisation 523
- Minims (unit eye drops) 193
- Mists, bactericidal 416
- Mixture of  
Ferrous Sulphate 549  
for Infants 549
- Magnesium Hydroxide 53
- Sodium Salicylate 549
- Mixtures 40, 49  
diffusible solids in 43  
precipitate forming liquids in 46, 51  
resinous tinctures 46, 51  
slightly soluble solids 47  
soluble substances 40
- Mode of action  
aerial bactericides 416, 417
- arsenicals 455
- dry heat 309
- ethylene oxide 518
- ionising radiations 530-1
- mercurials 455
- moist heat 316, 319
- sulphonamides 455
- ultra violet light 527
- Mont Granulation 98, 99
- Moisture damage to  
oily substances 313, 316  
powders 313, 316, 512
- Mop  
disinfectant soaked 408  
oil impregnated 407
- Morphine and Atropine Injection 268, 549
- Morphine Sulphate Injection  
instability 268, 276, 294, 549  
preparation 360, 386-7
- Mortar and pestle, sterilisation 313
- Mould  
bougie 172  
calibration 169  
lubrication 170  
suppository 169
- Moulded tablets (tablet triturates) 85
- Moulds  
contamination of media 230  
culture media for 235  
damage to medicaments 259, 265  
factors affecting growth 234-5  
heat resistance 234  
sterility tests 234  
thermal resistance 309, 316
- Movement  
excessive in asepsis 424, 453  
restricted 523
- M.S. 510/500/550 539
- Muctilage of Irish moss as emulsifying agent 55, 61  
of Tragacanth as suspending agent 45, 47, 48
- Muffle furnace 315, 481
- Muslin  
covering for plugs 313  
for clarification 366, 545  
wrapping for dressing 340
- Mycobacterium tuberculosis*, in milk 347, 401, 449
- NAILS, cleaning for asepsis 424, 426
- Nalorphine Injection 277
- Nandrolone Phenylpropionate Injection 244, 314
- Nasal  
bougie 168  
catheter 221  
inhaler 220
- Nebulae 181
- Needle  
air escape 433, 434, 505  
corrosion 538  
filling 370, 390, 503, 505  
for passing carbon dioxide 396  
gauge 433, 537  
sterilisation 312, 523, 533, 537  
sterility test 472  
stilette 538  
syringe  
aseptic handling 434  
blockage 270, 293  
cleaning 240, 538  
disposable 287, 537, 538  
for aqueous solutions 537  
for suspensions 537  
intravenous 302, 303  
non-disposable 537  
packaging 312, 433, 435, 537, 538, 540  
types 433  
transfusion bottle 300, 301
- Neosarsphénamine 455, 503  
Injection 400, 503
- Neostigmine Injection 277
- Neutralisation, bactericides in sterility tests 429, 434-7, 474, 475
- Neutralising agents, inhibition by 452, 454, 456
- Nikethamide Injection 359, 459
- Nitrogen—(contd.)  
in preparation air free water 244  
supply 392, 404, 405  
use in infra red vacuum oven 543
- Nitroglycerine tablets 97, 120
- Nitrous oxide medical gas 217
- Non-ionic emulsifying agents 56, 139
- Non staining iodine ointment 148
- Noradrenaline Injection 549
- Nordihydroguaiacol acid, as antioxidant 551, 552
- Normal Saline Solution 191
- Nylon 285, 286, 301, 302, 303, 342, 487, 513, 524  
air filter 369  
film for dressings 340, 519  
overalls 354  
reaction with bactericides 536  
syringes 536
- OCULENTA (eye ointments) 134, 160, 309-11
- Oestradiol Benzoate Injection 240, 244, 245, 314
- Oil in water emulsions 55, 67, 70, 71, 75, 136
- Oiling, floors 404, 407
- Oils  
action on rubber 294  
as injection vehicles 244, 390, 513, 538  
clarification 390, 490  
germical, for mops 408  
sterilisation 312, 313, 482, 490  
sterility tests 465
- Oily Cream (Hydros Ointment) 133, 134, 137, 157
- Ointment 133  
Ammoniated Mercury 135, 146  
aseptic filling 314, 511  
bases  
animal fats 154, 156, 159  
Benzoinated Lard 156, 553
- B.P. and B.P.C. 158
- Cetomacrogol Emulsifying Ointment 141, 160, 162
- Cetrimide Emulsifying Ointment 160, 162
- Emulsifying Ointment 135, 138, 139, 143, 160, 161, 162
- Eye Ointment Base 134, 160, 509
- hydrocarbons 158
- lanolin 136, 159
- lard 159
- macrogols 161, 162
- Paraffin Ointment 159, 162
- properties 153
- selection 153
- Simple Ointment 135, 160, 162
- soft paraffin 158, 162
- water miscible 160, 161
- Wool Alcohols Ointment 135, 137
- wool fat 136, 159
- Boric Acid 159
- Calamine 147, 159
- Capsicum 135, 160
- Cetomacrogol Emulsifying 157, 159
- 160

## INDEX

- Ointment—(contd.)**
- Cetrimide Emulsifying 157, 159, 160
  - Dithranol 159
    - Strong 159
  - Emulsifying 133, 135, 138, 143, 157, 159, 160, 162
  - emulsions 136
  - eye 134, 137, 509-11
  - Eye Ointments B.P. 134, 160
  - Gall and Opium 147, 159
  - Hamamelis 159
  - Hydrocortisone 159, 160
  - Hydrocortisone Acetate 159, 160
  - Ichthammol 159
  - insoluble substances in 146
  - iodine 147, 148
  - Iodine, Non staining 148
  - labelling 134
  - Macrogol 133, 161
  - metallic oleates in 148
  - Methyl Salicylate Compound 136
  - official 162
  - packing of 134
  - Paraffin 157, 158, 159
  - phenol in 156
  - preparation by
    - emulsification 136, 138
    - fusion 134
    - trituration 145
  - Salicylic Acid 160
  - Sample 135, 157, 158, 160
  - sterilisation 313, 314, 510
  - Sulphur 135 160
  - Wool Alcohols 18, 135, 157
  - Zinc 135, 160
  - Zinc Undecenoate 161
  - Oleated Mercury 148
  - O P (Original pack) 215
  - Ophthalmic solutions, filtration sterilisation 488, 490
  - Ophthalmoscopes, sterilisation 523
  - Organic matter, effect on bactericides 261, 519, 524
  - Osmotic pressure 246, 247, 255
    - blood plasma 247
    - effects on bacteria 224
    - relation to freezing point depression 250
  - Iachrinal secretion 247, 250, 257
  - measurement 246, 247, 250
  - unfavourable, in sterility tests 459
  - Quanabain Injection 355
  - Oven
    - dry 313, 362, 363, 376, 385, 481, 538
    - forced convection gas 542
    - hot air 309-16 310, 541, 542
      - air pocketing in 311
      - applications 312-6
      - breakages in 312
      - Browne's tube for 345
      - containers for 312
      - cooling 312
      - fan 311, 312, 542
      - for instruments 543
      - gas heated 311
      - heaters 310, 311, 312
      - heating up 311, 312
    - oven, hot air—(contd.)
      - lag times 311, 312
      - loading 311, 312, 314, 391, 541
      - method of use 311-12
      - opening of door 510
      - overheating 311, 312
      - temperature control 310, 311, 312
      - infra red conveyor 345, 541-2, 543
      - infra red vacuum 543
    - Overage
      - ampoule 374 386 389
      - powder ampoule 500
      - siliconed container 280
    - Overdosage 354, 393
    - Overseal 291, 372
      - cartridge 297
      - metal 293, 372
      - multi-dose container 385
      - plastic 372, 382, 385
      - transfusion bottle 372
    - Oxidation
      - by ozone 528
      - catalysis by rubber extracts 292
      - cutting instruments 313, 439, 543
      - death cause in dry heat 309
      - medicaments 391, 417, 481
      - of articles in dry heat sterilisation 319
      - prevention in injections 244, 267, 392, 393 563
      - rubber gloves 544
    - Oxidation reduction, indicator 231, 232
    - potential 223
      - of culture media 228, 231, 232, 429, 449 464, 465, 472
    - Oxidised cellulose 545
    - Oxophenarsine salts 455
    - Oxygen, effect in radiation sterilisation 531
    - Oxytetracycline, stabilisation 268, 458 475
    - Oxytocin Injection 265 276, 277
    - Ozone 527
  

**PACKAGING**

    - catgut 467
    - for asepsis 435, 528
    - for sterilisation
      - by dry heat 312, 313
      - by ethylene oxide 519
      - by ionising radiations 533
      - disposable syringe needles 537
      - dressings 319, 329, 339-41, 470
      - dusting powders 315, 507, 508
      - eye ointment tubes 313
      - filtration units 489
      - glassware 313, 319
      - glove powders 544
      - instruments 319
      - medical lubricants 314
      - membrane filters 484
      - powder funnels 330
      - rubber gloves 544
      - syringes 312, 472, 539-40
      - hypodermic tablets 545
      - implants 546
    - implants 546
    - Pain, from injections 265, 270, 276  

**Paints** 181-3

**Papaveretum, hypodermic tablets** 545

**Papaveretum Injection** 350, 361

**Paper**
    - air permeability* 341
    - compressed, for air filtration 414
    - ethylene oxide absorption 520
    - ethylene oxide permeability 519
    - glassine 341, 467, 470
    - Klontex 344
    - permeability to bacteria 341, 470, 519
    - pin holes 341
    - steam permeability* 341
    - steam sterilisation 341
    - tests for 341
    - water repellent 341
    - wrapping, for sterilisation 313, 340-1, 519
      - for light protection 284

**Para-amino benzoic acid (PABA)** 223, 455, 508

**Parafilm**
    - Gauze Dressing**, sterilisation 314, 316
    - sterility test** 471
    - hard 159
      - for closure sealing 293
    - Ointment** 156, 159
    - soft 156, 158, 162, 313, 314, 509

**Paraldehyde** 524

**Paraldehyde in mixtures** 48

**Paratonic solutions** 247

**Particle size**
    - aqueous injectable suspensions 270
    - in air filtration 411, 412, 415
    - only injectable suspensions 271
    - surphonamides 315

**Particles**
    - from filters 366, 367, 482
    - from rubber closures 294, 371
    - in air 400, 419
    - in injections 354, 362, 371, 381, 382, 385, 402
      - inspection for 370-1, 379, 380, 391
    - metal from eye ointment tubes 511
    - removal from eye ointments 510

**Partition coefficient, bactericides in rubber and water** 293, 294

**Paste**
    - Lassar's 133 162
    - Uma's 149, 162

**Pastes and jellies** 133, 149, 162
 
    - aluminium hydroxide in 152
    - bentonite in 152
    - cellulose derivatives in 151
    - gelatin in 149
    - macrogols in 161
    - pectin in 152
    - polyethylene glycols in 161
    - starch in 150
    - tragacanth in 151

**Pasteurisation** 347

**Pasteurisers** 347

**Pastilles** 198

**Pathogen** 222, 309, 310, 314, 316, 324, 343, 344, 347, 349, 400, 401, 415, 449, 472, 473, 542, 543

Pencils, glass writing 422, 423, 439, 462

Penicillin 279, 512-6

  containers 279, 512

dermatitis from 298, 409

effect of heat 315, 512

fermentation 413

hygroscopicity 410, 512

inactivation by penicillinase 259,

411, 513

intrathecal 515

metal traces on 268, 513

moisture sensitivity 292, 456, 507,

508, 512

preparations 457, 512-6

  Buffered Solution Tablets 515

  dusting powders 315, 400, 506,

508, 515

  Eye Drops 515

  Eye Ointment 513, 515

  hypodermic tablets 545

  injection, see Penicillin Injection

  lozenges 512

  ointment 512

  pyrogen in 364

  tablets 286, 512

  stability 265, 268, 512-3, 522

  sterility tests 446, 456-7, 460,

464, 467, 508

  storage 512, 513, 515

Penicillin injection

  buffering of 267

  container 277, 292

  decomposition by rubber 292, 513

  depot 249, 515-6

  preparation 505 513-5

  stability 305 512 3

Penicilline 259, 305, 411, 453, 456,

467

  activity 457, 513

  penicillin sterility test 446, 456-7,

458, 460, 464, 508

  preservative 457, 458, 509

  production 456-7

Peptone 226, 450

mycological 234

Percentage calculations 15

  acids 34, 38

  alcohols 32, 33

  miscellaneous 34

  v/v (volume in volume) 31, 32, 33

  v/w (volume in weight) 31

  w/v (weight in volume) 15

  w/w (weight in weight) 29, 30

Perfusion fluid, see Infusion fluid

Perspex, see Polymethyl methacrylate

Petri dish 312, 313, 473

Pessaries 168

  B P C and B N F 179

pH

  adjustment

    colorimetric 227-8, 266, 366,

    394-5, 397

    culture media 227-8

    electrometric 227, 266, 366, 395

  injections 266, 277, 366, 394-5,

397

  antagonistic to bacteria 224, 227,

317, 413

  barbiturates 454

pH—(contd)

  changes due to rubber 266, 292

  culture media 227, 413

  eye drops 184

  influence on heat sterilisation 265,

317

  injections 265, 266, 276, 277, 348,

366, 383, 395, 397, 561

  meter 227, 266, 366, 395, 396

  optimum for bacterial growth 421

  optimum for penicillinase activity

513

  stabilisation by 265-7, 383, 391,

392, 512, 561, 562

Phantom eyes 417

Phase volume ratio, emulsions 70

Phenobarbitone Injection 243, 499

Phenobarbitone sodium 454, 499-501

Phenol 361, 389, 390

  absorption by rubber 259, 293

  action on plastic surfaces 404

  antifungal activity 235

  bactericide in injections 259, 260,

261, 265, 389, 457, 460, 514, 557

  binding by plastics 286

  inactivation in sterility tests 454,

459, 462

  incompatibilities 261, 557

  Oily Injection 314, 389-91

  ointments 156

  smarting on injection 261

Phenols

  aerial bactericides 417

  asepsis laboratory disinfectants

408, 417

  inactivation in sterility tests 454

Phentholamine Injection 268, 393

Phenylethyl alcohol, in eye drops 189

Phenylmercuric acetate, eau de tubung fluid 467

Phenylmercuric borate 259, 349, 459

Phenylmercuric nitrate 259, 261, 348,

382, 383, 386, 394 395, 465

  absorption by rubber 293, 294, 383,

514

  antifungal activity 235, 259

  bactericide

    in injections 259, 294, 383, 514,

557

    in eye drops 189

  in Heating with a Bactericide 348

  inactivation in sterility tests 455,

459

  incompatibilities 350, 557

  sporocidal activity in oil 260

  sterilisation of implants 546

Phosphatase test of pasteurisation 347

Photochemical damage from ultra

violet 529

Physiological salt solution in eye drops 189

Phystostigmine Eye Drops 184, 190,

192, 193, 194

Phytomenadione Injection 240, 261

Picrotoxin Injection 277

Piercing machine, closure tests 295

Pills

Pills 93

  coating 94

  enteric coating 95

  excipients 95

  formulation 93

  homogeneity 93

  packing 95

  preparation 94

  shape 93

  solubility 93

  tastelessness 93

  varnishing 94

Pin holes, syringe containers 539, 540

Pipette

  bulb 384, 387, 394

  capillary 313, 394, 427

  graduated 313

  packaging for sterilisation 312, 313

  Pasteur 424, 426, 427, 429, 430,

432, 435, 464, 465

  sterilisation 312, 313

  rack 422, 423, 424, 427

  use for bacteria 463

Plasma 279, 282, 301, 528, 529, 532

Plasters 165

Plastics 284-7, 467

  additives 285, 287

  ageing 285, 286

  bags, for injections 286, 287, 303

  Bakelite 286

  bonding of medicaments & preservatives 285, 286, 299

  brittleness at low temperatures 286

  chemical resistance 285 286, 287, 404

  contamination level 521

  density 285, 286

  dimensional stability 285, 286

  dust attraction 285, 286, 493

  elasticity 306

  ethylene oxide damage 520, 523

  extractives from 286

  fabrication 285, 286, 287

  filtration media 367, 479, 483

  flexibility 285 286

  foam 385 406, 538

  for sterile containers 285 298

  for sterile equipment 285

  heat conduction 285

  heat sensitivity 285, 286, 287, 404,

423

  injection containers 285, 298

  labelling 285

  lamnates 404, 406

  lightness 407

  mechanical strength 285, 286, 287

  melamine formaldehyde 287

  microporous 483, 493

  moisture resistance 287

  oil resistance 285, 286

  overseals 372, 382, 508, 515

  permeability to

    bacteria 340

    bactericides 306

    flavours 286

    gases 285, 286, 287, 299, 519

    oils 285

    sodium metabisulphite 306

    steam 340

    water vapour 285, 286, 287, 299

- Plastics—(contd.)**  
 phenol formaldehyde 286  
 polyamide, *see* Nylon  
 properties 285  
 protection from light with 287  
 sachets for catgut 545  
 solvent resistance 286  
 sterilisation 285, 286, 287, 316, 342,  
 408, 517, 519, 520, 536  
 tainting of contents 285, 286  
 tests, on injection bags 287  
   for extractives 287  
   for pyrogens 287  
   for toxicity 287  
 thermoplastic 285  
 thermosetting 285, 286, 313  
 toxicity 285  
 transmission curves 287  
 transparency 285, 286, 287, 407,  
 409  
 types, *see* under individual names  
 urea formaldehyde 287  
 water absorption 286  
**Plug, cotton wool**  
 absorbent 334  
   bacteria from 429, 496  
   non absorbent 229, 301, 338  
**Plugging**  
 eye ointment tubes 511  
 filter holders 489  
 glassware 312, 313, 427, 435, 439,  
 488, 489  
 syringe containers 433, 540, 541  
**P M M A , *see* Polymethyl methacrylate**  
 Polishes, germicidal floor 408  
 Polishing, injection containers 373,  
 380, 382, 385  
**Polyethylene, *see* Polythene**  
 Polyethylene glycol (Macrogol), oily  
 injection vehicles 245  
 pastes 161  
 Polyethylene glycol derivatives as  
 emulsifying agents 139  
 Polymethyl methacrylate 285, 286,  
 407  
 Polymyxin B sulphate, sterility test  
 458, 475  
 Polypeptides, instability 265, 276  
 Polypropylene 286  
 Polystyrene 285, 286, 299, 373, 523,  
 524, 536  
 Polytetrafluoroethylene 285, 286, 436,  
 466, 490  
 Polythene 285, 286, 292, 299, 437,  
 484, 493, 519, 520, 522  
   high density 285, 368  
 Polyvinyl chloride 285, 286, 292, 302,  
 303, 342, 367, 403, 404, 489, 513,  
 520, 524  
 Pore, distribution 492  
   maximum size 475, 483, 492  
 uniformity of diameter 492  
 Posological tables  
   in ascending order 583  
   in pharmacopoeial order 575  
 Posology 573  
 Posterior Pituitary Injections 276, 358,  
 446, 482, 532  
**Potassium Chloride Injection, labelling**  
 357  
**Potassium permanganate, for filter**  
 cleaning 481, 482  
   in formaldehyde fumigation 524  
**Powders** 78  
   bulk 78  
   compound 79, 91  
   containers for sterile 306, 402, 443  
   containing liquids 90  
   double wrapping for 78  
   dusting 89, 314–15, 506–9  
   free flowing 506  
   granular effervescent 87  
   hydroscopic substances in 78  
   in cachets 83  
   in capsules 84  
   moisture sensitivity 313, 522  
   simple 78, 79  
   small doses in 81  
   standardised 90  
   sterilisation 311, 312, 313, 314–16,  
 519, 520, 522  
   thermolabile 400, 522  
   volatile substances in 78  
   wrapping of 78  
**Powdered Opium** 91  
**Precipitation, aseptic** 479, 499, 517,  
 519, 522  
**Precipitron** 415  
**Prefilters**  
   air filtration 334, 413, 414  
   liquid filtration 485  
**Preliminary compression, tablet making**  
 98, 101  
**Preparation, aseptic**  
   hypodermic tablets 545  
   implants 546  
   jellies and creams 546  
   lamellae 545  
   oily injections 314  
   penicillin products 315, 513–16  
**Prepared, Belladonna Herb** 91  
   Digitalis 91  
   Ergot 91  
   Ipecacuanha 91  
**Prescription** 354  
**Preservatives**  
   absorption by rubber 292, 295, 304  
   bonding by plastics 285, 286  
   in emulsions 69  
   in eye drops 188  
   in injections 258–61  
   in ointments 156, 157  
**Pressure**  
   absolute 318  
   gauge 318, 327, 328, 332, 343  
**Pressurisation, asepsis laboratory** 403,  
 421  
**Procainamide Injection** 259, 549  
**Procaine hydrochloride** 250, 251, 252,  
 254, 266, 277, 388, 515  
**Procaine and Adrenaline Injection**  
 266, 268, 277, 349, 388, 549  
**Procaine benzylpenicillin** 270, 456,  
 515, 520  
   Injection, aqueous 270, 271, 280,  
 292, 515  
   oily 271, 314  
**Processing, aseptic** 266, 412, 453, 479–  
 516, 529  
**Progesterone Injection** 240, 244, 245,  
 314  
**Promazine Injection** 244  
**Proportional calculations** 37  
**Proprietaries**  
   containers for 215, 216  
   dispensing 215  
   Draft Code on Dispensing of 215  
   labelling 215, 216  
**Propyl gallate** 551, 552, 553  
**Propylene glycol**  
   aerial bactericide 417  
   injection vehicle 245, 246, 349  
**Propylidone Injections** 240, 314, 463  
**Protamine Zinc Insulin Injection** 267,  
 463  
**Protection, post sterilisation** 339, 340,  
 341  
**Protective colloids, in mixtures** 51  
**Proteins, protection by in sterilisation**  
 317, 524, 537  
**Pseudomonas species** 223, 451, 484  
 555  
**Psychotroph** 224, 451  
**P F F E , *see* Polytetrafluoroethylene**  
**Pulv pro mist** 52  
**Pump, vacuum**  
   hand 482, 485  
   oil-sealed 331, 332, 334, 336, 338,  
 342, 369, 404, 405, 485, 491, 492,  
 520  
   water jet 369  
   water ring 332  
**P V C , *see* Polyvinyl chloride**  
**Pyndostigmine Injection** 261  
**Pyrogens** 240–3, 366, 396  
   dry heat destruction 316, 362, 555  
   films on glassware 242, 362, 555  
   in medicaments 364  
   production 348, 362, 364, 366, 369,  
 481  
   quantitative estimation 555  
   removal by, adsorption 364  
   ion exchange resins 243  
   resistance to 555  
   sources 241, 555  
   standard preparations 555  
   tests 243, 554–6  
    on giving sets 287, 303  
  
**Q F EMULSIFIER** 74  
**Quantum** 526, 531  
   energy 526, 527, 529  
**Quaternary ammonium compounds**  
   asepsis laboratory disinfectants 408  
   emulsifying agents 56, 139, 144  
   inactivation in sterility tests 455,  
 456, 475  
**Quinine and Urethane Injection** 249,  
 265  
**Quinine Hydrochloride Injection** 249,  
 317  
  
**RABBITS, for pyrogen test** 554, 555, 556  
**Rad** 531

- Radiation, *see* Heat transfer  
 Radiation, thermal 541, 542  
 Radiation, sterilisation 526, 529-33  
 Radiations, electromagnetic  
     infra red 526, 541  
     ionising 526, 529-33  
         deleterious effects 530, 532  
         from isotopes 529, 533  
         machine generated 529, 530, 533  
         mode of action 530-1  
         penetrating power 529, 530, 532  
         pulsed 530 533  
         sterilisation by 309, 467, 529-33,  
             544  
         wavelengths 526  
 Radiations, particulate  
     alpha 526  
     beta 526  
 Radiators, air disturbance from 410,  
     421, 529  
 Radioactive isotopes 472, 527, 530,  
     531  
 Radioactivity, unit of 530  
 Rancidity, injectable oils 244  
 Range, ionising radiations 529  
 Rationalisation of weights and mea-  
     sures 9  
 Reactivation, metabolic of bacteria  
     527  
 Recirculation, in air ventilation 421  
 Rectified spirit 31  
 Reducing agents 549  
     in culture media 228-9, 231-2, 449,  
         450  
     in injections 267, 268, 392, 562  
 Reducing valve, *see* Valve  
 Refrigeration 265, 361, 512, 513, 515  
 Regulators, gas cylinder 279  
 Refajel labelling equipment 361  
 Removal, aseptic  
     Pasteur pipette from tube 427  
     syringe from container 539  
     tube caps 427  
 Resazurin 232, 449  
 Resin, treatment of air filters 413, 414,  
     419  
 Resinous tinctures, in emulsions 58  
     in mixtures 46, 51  
     list of 47  
 Resorcinol aerial bactericide 417, 418  
 Respiratory tract, organisms from  
     401, 408, 409, 415, 416, 420  
 Revision exercises  
     emulsions 76  
     incompatibilities 213, 214  
     liniments 131, 132  
     lotions 131, 132  
     mixtures 54  
     ointments 163  
     pastes 163  
     powders 92  
     suppositories 179  
 Rider, use of 384, 394  
 Ringer Lactate Injection 396  
 Rinsing  
     glassware 362  
     hands 425  
 Rotary capsule machine 197  
 Rotary tablet machine 107, 108  
 Routes for injection 237, 238, 239  
 Rubber  
     absorption of  
         bactericides 189, 259  
         water 289, 290, 291  
     accelerators 288, 289, 290, 292  
     activators 289, 292  
     ageing 289, 290, 291, 296  
     antibacterial extracts from 229, 230  
     antioxidants 289, 290, 291, 292  
     butyl 290, 292, 306  
     chemical resistance 289, 290  
     chloroprene 291, 313, 391  
     cleaning agents on 363, 544  
     colouring agents 289, 292, 295, 363  
     compound 288, 289, 363  
     elasticity 288, 291, 301, 306  
     extraction from injections 292  
     extractives 229, 230, 266, 291, 296,  
         363, 292  
     fillers 289, 290, 292  
     hardness 289, 290, 291  
     heat resistance 288, 290, 291, 313,  
         314, 338, 391  
     Hycar 290  
     inactivation of injections 292  
     latex 288, 290, 291  
     liquid properties 293, 294  
     lubricants 289, 292, 294, 363  
     manufacture 289  
         of articles 289-90  
     natural 288, 292, 296, 369, 391  
 Neoprene 291  
 nitrile 290  
 oil resistant 290, 291, 294, 391  
 oxygen resistance 289, 291  
 ozone damage 528  
 pale crêpe 288, 291, 294  
 particles in injections 371, 438  
 permeability to  
     gases 291, 292, 306, 519  
     water 291, 306  
         water vapour 290, 292, 296  
 pharmaceutical 291  
 silicone 230, 280, 291, 292, 313, 338,  
     367, 391, 437, 448, 488  
 smoked sheet 288, 294  
 softeners 289, 290, 292  
 solvent resistance 288, 290  
 sterilisation 291, 313, 316, 342, 489,  
     517, 520  
 stickiness 363, 364  
 sulphur compounds from 363  
 synthetic 290-1, 294, 306, 369, 391  
 toxicity 291, 292  
 vulcanisation 288, 289, 291, 292  
     cold curing 288, 292  
     heat 288, 292, 294  
     zinc from 292  
 Rusting, of instruments 316  
 SAMPLING of air, by 418-9, 420, 447,  
     453, 484  
 bubbler 419  
 electrostatic samplers 419  
 filters 419  
 settling plates 418, 543  
 size grading sampler 419  
 Sampling of air, by—(contd)  
     slit sampler 418, 419, 420  
     Wells air centrifuge 418  
 Saponin emulsions 55, 56  
 Saprophytes 310, 448, 451  
 Saturated solutions 124  
 Schick Test Toxin 250, 261  
 Sclerosing agents 249  
 Screen, asepsis, 406, 407, 421, 422,  
     423, 427, 435, 437, 440, 450, 453  
     air supply to 421  
     ethylene oxide 523  
     fume-cupboard type 407, 437  
     plastic 286, 407  
     shack type 407, 422, 437  
     tall, for sealing 436, 442, 502, 503  
     tidiness 431, 435  
     ultra violet lamp in 415, 528  
 Screens for granulation 98, 99  
 Scrubbing up 403, 424-425  
 Seal  
     aluminium ring  
         antibiotic vial 305, 513  
         Climbric bottle 305, 306  
     paraffin, for culture media 232  
     plastic ring 372, 508, 515  
         Climbric bottle 305, 372, 441  
         culture media 230  
         transfusion bottle 372  
     rubber test tube 229  
     syringe tube 539, 540  
     Visking, *see* Seal, plastic ring  
 Sealing:  
     ampoules 378-9  
         aseptically 436  
     Climbric bottle Mark I aseptically  
         440, 441  
     floors 404  
     metal dressings drums 341  
     nitrogen filled ampoules 393, 503  
     ointment tubes aseptically 546  
     packaging films 373  
     paraffin gauze dressing tin 314  
     powder ampoules 502, 503  
     rubber closures 293  
 Seats, aseptic laboratory 406  
 Sedimentation rate  
     air particles 401, 418  
     injectable suspensions 270, 393, 463  
 Seidite Powder 92  
 Self-emulsifying monostearin 139, 140  
 Self-sterilising fluids for eye drops 190  
 Semi-permeable membranes 246, 247  
 Sensitisation by  
     cetrimide 423  
     penicillin 513  
     skin bactericides 424  
 Separator, steam steriliser 324, 332  
 Sequestring agents  
     for cleaning 362, 363  
     in injections 267, 268, 513  
     in rubber 289  
*Serratia marcescens* 420, 491  
 Serum 296, 301, 476, 488, 519  
 Services, asepsis laboratory 404-6,  
     422  
 Sesame oil, injection vehicle 244, 260,  
     271, 314  
 Sheetng, rubber 290, 339

- Shelf life, injections 265, 499, 512  
 Shoes, asepsis laboratory 407, 409  
 Sieves, for granulation 98, 99  
 Sight glass, steam steriliser 333  
 Silicone  
 cream 145  
 glass treatment 279, 280  
 grease 471, 488  
 syringe lubricants 536, 538, 539, 541  
 Silver nitrate, adjustment to isotonicity 258  
 Silverson emulsifier 73  
 Simple Cream 139  
 Simple Ointment 135  
 Sintering 479, 480, 481, 483  
 Skin  
 antibacterial activity 402  
 bacterial flora 401-2, 408, 425  
 disinfection 423, 424-6  
 protection from ultra violet 409  
 Slag wool 413, 414, 421  
 Slightly soluble liquids in mixtures 47  
 Sludging 98, 101  
 Small quantities, to obtain  
 from standard solutions 49  
 in mixtures 49  
 in pills 96  
 in powders 81  
 Small quantities, weighing 39  
 Snuff 39  
 Soap  
 effect on activity of bactericides 423, 456  
 emulsions 56, 63-6, 137  
 filtration 494  
 green soft 537  
 hexachlorophane 424, 425  
 liniment 18, 130  
 liquid 405, 537, 544  
 Sodium alginate, air filter 419  
 Sodium Aurothiomaleate Injection, labels 359  
 Sodium Bicarbonate Injection 269, 357, 364, 395-6  
 Sodium carboxymethylcellulose, suspending agent 270  
 Sodium chloride 248, 339, 355, 396, 420, 443, 453, 464  
 adjustment to isotonicity with 250, 252, 254, 395  
 in culture media 449  
 pyrogen in 316, 364, 365  
 sterilisation 315  
 Sodium Chloride Injection 245, 250, 254, 545  
 glass flakes in 278  
 immediate sterilisation 241  
 labelling 357, 359, 361  
 Sodium Chloride and Dextrose Injection 241, 250, 278  
 Sodium citrate 267, 278, 366, 443, 464, 515  
 Sodium hexametaphosphate, sequestering agent 268, 362, 537  
 Sodium hydrosulphite 449, 450  
 Sodium hypochlorite, aerial bactericide 417, 418  
 for syringe cleaning 537  
 Sodium lactate, pyrogen in 364  
 Sodium Lactate Injection, 241, 278, 357, 366  
 Compound 241, 250, 265, 278, 357, 396  
 Sodium lauryl sulphate, quat antagonist 436  
 Sodium metabolysulphite, reducing agent 267, 268, 276, 294, 355, 386, 388, 345, 549, 557, 562  
 Sodium Morrhuate Injection, sclerosing agent 249  
 Sodium Salicylate Mixture, Strong, *B N F* 549  
 Sodium sulphite, reducing agent 562  
 Sodium thioglycollate 449, 450  
 arsenicals sterility test 455  
 inactivator of streptomyces 457  
 inhibitory concentration 455  
 neutraliser of mercurials 455  
 Soft gelatin capsules 196  
 manufacture 197  
 seamless 197  
 Soil  
 for testing sterilisers 343  
 source of dry organisms 467  
 Solids for injection 268, 446, 475  
 glass for 277  
 packaging 479, 499  
 preparation of injections 513, 515  
 Solubility  
 bactericides in air 416  
 changes from radiation sterilisation 532  
 hypodermic tablets 545  
 Soluble solids in mixtures, list 40  
 Solution for Eye Drops 189  
 Solutions of Ferrous Iodide, reducing agent in 550  
 Solutions  
 colligative properties 247  
 concentration during sterilisation 326  
 hypertonic 247, 248, 249, 250, 513  
 hypotonic 247, 249, 250, 397  
 iso-osmotic 247  
 isotonic 247, 264, 397, 512  
 ophthalmic 184  
 paratonic 247, 249  
 saturated 124  
 Sorbitan esters, as emulsifying agents 139  
 Spans 140  
 Specific gravity, of injections 269  
 Spicules, glass  
 from equipment 365  
 from opening implant tube 546  
 in ampoules 371, 376, 435, 514  
 inspection for 371  
 Spores bacterial 222-3, 321, 322, 349, 449, 471  
 in air 400, 413, 415, 418  
 in catgut 467  
 chemical resistance 222, 259, 260  
 contaminants of vaccines 347  
 dessication resistance 222  
 destruction in Tyndallisation 348  
 ethyl alcohol on 425  
 ethylene oxide on 518, 521  
 Spores bacterial—(cont'd)  
 for testing steriliser efficiency 343, 344, 521, 522  
 heat resistance 222, 224, 316, 319, 324, 345, 542  
 in dusting powder ingredients 314  
 optimum growth conditions 447  
 survival in pasteurisation 347  
 radiation resistance 531  
 removal from ampoule exterior 430  
 skin disinfectants on 426  
 soil 542  
 ultra violet resistance 527  
 Spores, mould  
 in air 401  
 moist heat resistance 316  
 ultra violet resistance 527  
 Spores  
 test papers, 344  
 Oxid 344  
 Spraying, aerial bactericides 416-8  
 Spray solutions 181  
 Sprays, reducing agents in  
 Adrenaline and Atropine, Compound 549  
 Isoprenaline 549  
 Isoprenaline, Compound 549  
 Stabilisation  
 aerial bactericides 417  
 formaldehyde solution 524  
 injections 265-9, 283, 393, 560  
 Stainless steel  
 aseptic equipment 407, 422, 481, 490, 511, 513  
 bench tops 404  
 Standard measures 9  
 Standardisation, compressed tablets 109, 110, 111, 112, 113, 114, 115  
 Standardised powers 90  
*Staphylococcus aureus* 317, 347, 401, 402, 449, 460, 463  
 Oxford H strain 460, 463  
 Starch  
 emulsions 52  
 pastes 150  
 Steam  
 dry saturated 318, 321, 543  
 drying of 332, 336  
 ejector 330  
 mixed with air 318, 322-4, 329, 546  
 saturated 318, 319-20, 322  
 advantages in sterilisation 319  
 condensate from 320  
 heat content 319, 336  
 liberation of latent heat by 319, 320  
 penetration 320, 323, 324, 331, 339, 340, 544  
 volume contraction 320  
 superheated 318, 319, 320-2, 330  
 causes 320-21  
 in large sterilisers 320, 321, 323, 343, 346  
 in portable sterilisers 320, 326  
 tolerable degrees of 321, 322  
 under pressure 316, 317, 318  
 dryness fraction 324  
 M.R.C. recommended exposure, 325

- Steam, under pressure—(contd)  
 principles of sterilisation by 318–  
 25  
 wetness 320, 321, 324, 332, 334,  
 336, 340  
 wet saturated 318
- Steam trap  
 balanced pressure 329, 330, 332,  
 333, 337  
 ball float 336, 337  
 near to steam 330
- Steamer 349
- Steaming 316, 317, 387, 389, 484
- Sterilisation, immediate 241, 242, 244,  
 397
- Sterilisation methods  
 Autoclaving, *see* Heating in an Auto-clave  
 by radiations 526–33  
 chemical 309, 344  
   gas, *see* Sterilisation methods  
   gaseous  
   liquid 309, 529  
 classification of injections under  
 558 9  
 dry heat 309–16, 344, 400, 440, 539,  
 555  
   advantages and disadvantages  
 316  
   in muffle furnace 315  
   penetration in 320, 522  
   mode of action 319  
 ethylene oxide  
   gaseous 517–24  
   liquid 546
- Sterilisation by Filtration 259, 309,  
 380, 386, 396, 400, 404, 435, 479–  
 99, 512  
   advantages and disadvantages  
 492 3  
   alternative to a heating method  
 374  
   aseptic technique in 446, 492  
   bactericides in 259, 493  
   emergency use 492, 493  
   mechanisms 493–4  
   sterility tests following 446, 493,  
 494, 496–8  
   virus removal in 493
- flaming, *see* Flaming
- formaldehyde 524
- gamma rays 286, 529–33, 532, 536,  
 537
- gas oven 542
- gaseous 499, 517–24, 545
- heat 309–50, 447
- high speed electrons 529–33
- H.T.S.T., *see* Pasteurisation
- infra red 345, 526, 541–2, 543
- ionising radiations 309, 467, 524,  
 528, 529–33, 544
- moist heat 309, 316–50
- pasteurisation 347
- physical 309
- steam under pressure 318–46, 524  
   applications 338–42, 407, 414,  
 438–9, 541, 544, 546
- Tyndallisation, *see* Tyndallisation
- ultra violet light 309, 527–9
- Sterilisation of:  
 absorbable haemostats 545  
 air  
   by chemicals 415–8  
   by electrostatic precipitation 414  
   –5  
   by filtration 411–14, 415, 528  
   by heat 411, 415, 420  
   by ultra violet light 415, 528  
   evaluation of techniques 418–21
- ampoules 361, 379, 430
- bacterial filters 484, 493
- blankets 517
- blood products 484
- bottled fluids 318, 322, 335, 338–9
- cauter 467–8, 530, 533, 544–5
- catheters, plastic 523, 533
- cellulose film envelopes 539
- closing tool 441
- closures 291, 338, 431, 439
- containers 285, 299, 303, 304, 338,  
 350, 439, 522
- creams in tubes 546
- crystals 519
- culture media 230, 348, 476, 546
- disposable giving sets 303
- distribution units 438–9
- dressings 339–42, 442, 466, 533
- electrodiagnostic equipment 523
- equipment 309, 316, 518, 521, 523,  
 535
- eye drop bottles 492
- fibrin foam 545
- filtration units 489–90
- filter presses 487
- gelatin sponge 316, 545
- glass wool filters 414
- glassware 310, 312 3, 316, 338, 442,  
 443
- glove powders 544
- implants 546
- instruments 309, 316, 543, 544
- jellies in tubes 546
- magnetic stirrers 523
- membrane filters 484
- moisture sensitive substances 316,  
 523
- mortars and pestles 507
- needles 312, 523, 533, 537, 541–3
- nitrogen filter 503
- oils 313–4, 490
- oily solutions 310, 313–4, 389, 391,  
 490
- ointment bases 313–4, 510
- ointment tubes 313, 546
- oxidised cellulose 545
- pharmaceutical preparations 309
- plastics 285, 286, 522, 523, 533, 536,  
 537
- powder dredgers 507
- powders 314–6, 522
- rubber gloves 544
- rubber tubing 338, 519
- scalpels 313, 533
- scissors 313, 439
- slag wool filters 414
- solutions of extreme pH 317
- stainless steel articles 313
- sulphonamides 315, 507
- Sterilisation of—(contd)  
 sutures 467–9, 530, 533, 544–5
- syringe containers 539–40
- syringes 309, 310, 311, 312, 313,  
 316, 323, 526, 533, 535–43
- thermolabile equipment 517, 523,  
 533
- thermolabile medicaments 499, 517,  
 523, 528, 529
- tiles 422
- vaccines 346–7, 529, 533
- vegetable infusions 348
- Steriliser  
 conducted heat 542 543
- electric boiling water 349, 350
- ethylene oxide 520–1
- formaldehyde 524
- steam 325–38  
   automatic 337–8  
   large 320, 323, 324, 325, 328–38,  
 333, 342, 543  
   baffle in 329  
   by pass in 330  
   discharge channel in, *see* Discharge channel  
   downward displacement type  
 329–30, 335–6, 339, 342, 343  
 543  
   for bottled fluids 335–7, 339, 546  
   for surgical dressings 328–35  
   high vacuum type 331, 332,  
 336, 340, 342, 343 543  
   reducing valve in 320  
   sensing temperature in 333  
   separator in 332  
   steam ejector in 330  
   strainer in 330  
   vertical 339
- portable 318, 320, 322, 323, 324,  
 325–8  
   'Portable' 325, 326
- pressure-controlled 325–6
- temperature controlled 326–8
- T.R.F. 326, 327, 328
- Sterility tests 310, 446–77, 479  
 accidental contamination in 344,  
 430, 434, 447, 469 523
- after bacterial filtration 494
- air for 410 450
- as steriliser efficiency controls 342
- B.P. 446 447
- B.P. Addendum 1964 446
- B.P.C. for dressings 469–71
- closed screen technique 523, 524
- controlling bodies 446
- controls 450, 452–3, 458 464, 498,  
 503
- bacteriology laboratory for positive 453
- choice of organisms for 458, 463
- inoculation with organisms 426,  
 457, 463
- known sterile dressings as 469, 471
- negative 452
- on auxiliary substances 453, 466
- on technique 453
- on working conditions 453
- positive 453, 459, 467, 472, 476
- separate for joint media 462

## INDEX

## Sterility tests—(contd)

creams 464-5  
 culture media for 230-5, 448-50,  
 451, 458, 471, 496, 497  
 cooling before use 471  
 excessive dilution 451  
 joint 451, 468, 476, 496, 497  
 mycological 235, 476  
 pretreatment of anaerobic 462, 498  
 sample inoculation into anaerobic  
 428, 463, 465  
 sensitivity 447  
 separate aerobic and anaerobic  
 459, 470, 472, 475, 496, 497  
 viscosity 471  
 volume 451, 455, 458, 459, 462,  
 475, 496  
 design 446, 476  
 dispersal of samples 450  
 effect on product issue 447, 498  
 empty containers on 472  
 ethylene oxide technique 523  
 examination of 451  
 eye ointments 465-7, 511  
 factors affecting bacterial growth and  
 224  
 filtration method 458, 464, 466,  
 474-5, 482, 484, 523  
 for aerobes 451, 462  
 for anaerobes 451, 462  
 for antibiotics 475  
 for barbiturates 475  
 for catgut 446, 467-9  
 heat sterilised products 446, 447  
 immunological preparations 472  
 implants 446, 474  
 incubation conditions 223, 343, 451,  
 458  
 information given by 447  
 inhibitors in 429, 446, 453-8  
     dilution and neutralisation 457  
     dilution of 453, 459  
     neutralisation 453, 454-7, 459,  
     468  
     separation from 457-8  
 filtration sterilised injections 259,  
 459  
 hypertonic injections 459, 464  
 injections sterilised by Heating with a  
     Bacillus 439  
 interpretation 343, 446, 451-2, 469,  
 476  
 loss of sensitivity 454, 455, 458,  
 474  
 mixing of sample and medium 450,  
 463  
 multi-dose injections 432, 459, 460  
 mycological 475  
     U.S.P. 234, 475  
     W.H.O. 476  
 needles 472  
 non-inhibitory substances 446, 459  
 official requirements 448, 451, 452,  
 475, 476  
 oils 260, 465-7, 475  
 ointments 233, 465-7, 511  
 on aqueous solutions 458-63, 475  
 on bulk preparations 447, 451  
 penicillin 453, 456-7, 515

## Sterility tests—(contd)

powders, insoluble 464, 474  
     soluble 463-4  
 predilution 454, 457  
 rejection of sterile batch 450  
 repeats 448, 451, 452, 467, 469, 470  
 results, interpretation 451-2  
     reading of 459, 463  
     table of 458, 460, 497, 504  
 sampling 343, 447-8, 451  
     aseptically processed products 448  
     from bulk 446-7  
     official recommendations 447-8  
     powders 451, 503  
     random 447, 452  
     representative 463  
     rules 448  
     sizes 448, 451-2, 457, 462, 465,  
     466, 496  
     statistics 448, 452, 464, 469  
     steam sterilised products 448  
     suspensions 451, 463  
     volumes 451, 458, 462  
     weights 451, 458  
 shaking of 464, 465, 508  
 single-dose, large volume, injections  
 432, 459  
 single-dose, small volume, injections  
 430, 459  
 streptomycin 457-8  
 subcultures 344, 426, 455, 459, 463,  
 464, 465, 466, 471, 475, 502  
 surgical dressings 233, 469-71  
 suspensions 463, 465  
 sutures, see Catgut  
 syringes 474, 472  
 T.S.A. Regulations 446-7  
 test papers and pieces 343, 344, 522  
 testing procedure 451-2  
 transfusion assemblies 472  
 turbidity in 459, 464, 475, 502, 508  
 ultra violet light for 450  
 unfavourable osmotic pressure 459  
 viral 476  
 W.H.O. 446, 476  
 yeast 475-6

Subophen Injection 267, 268, 277,  
 382, 383, 384  
 Stilette, needle 437, 489, 538  
 Stills, Water for Injections 242  
     head for 241, 332  
 Stirrer, magnetic, oil sterility tests 466  
     plastic, injection suspensions 393

Stock

- bottles 3
- mixtures 52
- solutions 52

Storage

- bacterial filters 481, 483, 484, 493
- Browne's tubes 345
- injections 276, 307, 366, 373, 499,  
     512, 513
- containers 278, 363
- medicaments 365
- penicillin preparations 512-5
- pH changes from faulty 265
- rubber closures 385
- sterile dressings 339, 342
- materials 402

## Strainer, steam steriliser 330, 343

Streptodornase, sterility test 446  
 Streptokinase, sterility test 446  
 Streptococcus, beta haemolytic, in air  
 401  
*Streptococcus progenit* 449  
 Streptomycin 298, 364, 413, 457,  
 522  
     Injection 239, 277, 409  
     sterility test 457-8, 475  
 Subcultures, see Sterility tests  
 Sublingual tablets 97  
*Sulpharsphenamine*, sterility test 435  
 Sulphonamides

- charring in ampoules 377
- dusting powders 315, 400, 506
- eye ointments 509
- injections 243, 268
- sterilisation of powders 315
- sterility tests 455-6, 459, 463, 508

Sulphur dioxide, reducing agent 549

Sulphuring 279

Sulphydryl compounds inactivators in  
 sterility tests 455, 457

Sunlight, action on micro-organisms  
 527

Suppository bases

- absorption from 168
- aqueous 166, 167
- beeswax in 151
- cocoa butter 166
- emulsifying 166, 167
- glycerine-gelatin 167
- Imhausen (Witepsol) 167
- macrogol 167
- Massuprol 167
- oily 166
- properties of 166
- theobroma oil 166
- Witepsol 167

Suppository moulds

- calibration 169
- furnication 170
- sizes 168

Suppositories containing

- balsam of Peru 176
- chloral hydrate 174
- insoluble solids 171
- liquids 175
- semi-solids 174
- soluble solids 173

Suppositories made by cold compres-  
 sion 178

Suppositories of

- Aminophylline 178
- Bismuth Subgallate 171
- Bismuth Subgallate, Compound  
         171
- Cinchocaine 171
- Glycerin 177, 178
- Glycerin Soap 178
- Hamamelis 178
- Hamamelis and Zinc Oxide 178
- Hydrocortisone 179
- Morphine 179

Surfaces

- asepsis laboratory 403
- bench 402, 403, 404, 406, 422
- plastic 287, 325, 354

- Surface active agents  
antagonists for quats 456  
filtration of 494  
for syringe cleaning 537  
in oil and ointment sterility tests 466
- Suspending agents, in mixtures 45
- Suspensions, injectable 270-1, 475, 493  
aqueous 270-1, 400  
cartridge for 298  
deposits from in syringes 537  
needles for 537  
oily 271  
stability 270-1, 400  
sterility tests 450, 451, 472  
therapeutic 271
- Sutures  
absorbable 467  
non absorbable 469
- Suxamethonium Chloride Injection 277
- Swabbing  
bench top 422, 423  
caps of multi-dose injections 258  
effect on accuracy of weighing 423, 443
- exterior of ampoules 505  
screen 423
- Switch  
barometrically compensated 337, 338  
vacuum 343
- Symbols, for weights and measures 14
- Synergists 551
- Syringe  
air bubbles in 536  
assembly 539  
barrel 536, 537  
calibrations 537, 538, 540  
cement 535  
cleaning 240, 317, 535, 536, 537-8  
containers 539-41  
aluminum tube 540, 542  
caps for 540  
cleaning 540  
envelopes 539  
glass tube 542  
nylon film 539  
opening 540  
plastic ring for 540  
shaped 540  
slitters for caps 540, 541  
springs for 540  
steam permeability 541  
test tubes 539  
transparency 539
- corrosion 535
- decontamination  
after isotopes 543  
after pathogens 543
- deposits in 538
- discoloration 536 538
- lubrication 316, 538-9, 541
- needle mounts 535, 537, 538
- packaging 533, 539-41
- plungers 535, 536  
seizing of 536, 537, 538, 539, 540
- pyrogen from 555
- Syringe—(contd.)  
releaser 537  
replacement of parts 536  
scratching of 538  
services 535, 542  
sterilisation, see Sterilisation of syringes  
sterility tests 447, 472  
types  
all glass 472, 513, 535, 536  
cartridge 297, 298  
disposable 285, 286, 299, 524, 536, 543  
half Record 472, 535, 536 542  
insulin 537  
interchangeable 535  
Luer 433  
non-disposable 535, 536  
nylon 286, 536  
plastic 285, 286, 299, 472, 536  
radioactive isotopes 543  
Record 433, 472, 535, 536, 542  
tuberculin 537, 543  
use for, ampoule filling 378  
ampoules filled just before use  
505
- Syrup of, reducing agents in,  
Blackcurrant 549  
Ferrous Iodide 550  
Raspberry 549
- TABLET triturates 85, 545
- Tablets, see Compressed tablets & Moulded tablets  
hypodermic solution 545  
moulded 85, 545  
paraformaldehyde 524
- Talc  
flow properties 314  
glove powder 544  
pathogens in 314  
sterilisation 311, 314, 519, 522  
tissue reactions to 315, 544
- Taps, filling and filtration units 369  
water  
elbow-operated 405  
foot-controlled 405  
knee-operated 405
- Target theory 531
- Tears, osmotic pressure 184, 191
- Teats, rubber 289, 422, 427, 428
- Technique, non-touch 409, 426, 435, 441
- Temperature control  
asepsis laboratory 404, 410  
ethylene oxide steriliser 520  
steam steriliser 343
- lethal 224  
measurement, pyrogen tests 554  
on aerial bactericides 416 417, 420  
optimum for bacterial growth 223-4, 421  
pressure relationship 318  
records 343, 521  
sensing 343, 542
- Terrazzo for asepsis laboratory 403, 404, 407
- Test, chamber, aerial bactericides 420  
object, performance characteristics 345  
papers, see Spore papers  
pieces 521, 522  
tablets 344
- Testosterone  
Implants 474  
Phenylpropionate Injection 244, 314  
Propionate Injection 240, 244, 245, 314
- Tests for  
aerial bactericides 420  
air filters  
bacteriological 419-20  
methylene blue 420  
sodium flame 420  
alkalinity 276, 280-3, 297, 363, 374, 383, 386, 388, 392, 494  
B P 281  
B S for ampoules 282  
crushed glass 279, 280-1, 282  
injections requiring glass complying with 560-1  
International Pharmacopoeia 281-2  
narcotine hydrochloride 283  
siliconed containers 281  
sulphured glass 279, 281, 282  
U.S.P. 282  
weathered containers 281  
whole container 281, 282
- ampoule faults 371-2, 387  
aseptic technique (dummy runs) 447
- B S transfusion bottle 300  
closures B S 294-6  
ethylene oxide steriliser 518, 521-2  
fermenter air 420-1  
liquid filters, bacteria proof 480, 490-2  
bacteriological 490, 491, 493  
B P C 491  
bubble pressure 490, 491-2  
flow rate 491  
for membrane filters 491  
pore size 481 491-2, 493  
water permeability 492
- pasteurisers 347  
pressurisation of asepsis rooms 432  
pyrogen 364, 554-6  
rubber glove perforations 544  
steam sterilisers 342-6, 447  
sterility 446-77  
toxicity  
disposable giving sets 287, 303  
immunological products 473  
plastic bags for injections 287
- Tetanus 314 452
- Tetracycline 265 285, 458 475, 522
- Injection 458
- Theobroma oil, suppository base 166
- Thermal death times  
in dry heat 309-10  
in moist heat 316-317, 324 337, 345 346
- Thermistor, for pyrogen test 554

- Thermocouple for hot air oven 311  
pyrogen test 554  
steam steriliser 326, 333, 343  
vapour pressure determination 257
- Thermometer for hot air oven 311, 312  
pyrogen test 554  
steam steriliser 322, 326, 329, 333, 342, 343
- Thermophiles 223, 316, 324, 343, 344, 347, 348
- Thermosat, autoclave 326-7, 343
- Thiomersal 235, 259, 268, 269, 455
- Thiopentone Injection 237, 244, 282, 454
- Thiourea 268, 533
- Thixotropy 271
- Throat paints 181, 182
- Thrombophlebitis, from rubber extracts 292, 302
- Thrombosis, from intravenous oils 240
- Tidiness  
in asepsis 431, 435  
in injection preparation 354
- Tiles  
floor 403, 404  
for asepsis 313, 422, 423, 435  
for injection preparation 355
- for pH adjustment 395, 397  
wall 404, 405
- Tolerances in dispensing 5
- Topical products, glass for 282
- Toxicity of  
aerial bactericides 246, 416, 417, 418  
bactericides for injections 259, 260, 348, 361, 423, 424  
borate buffers 267  
dyes for ampoule testing 371  
gaseous sterilising agents 520, 524  
glove powders 344  
plastics 285, 302  
rubber extractives 292, 295  
stabilisers 268  
sulphonamides 315  
vehicles for oily injections 245
- Towels 339, 405, 426
- Tragacanth  
in emulsions 61  
in pastes 151
- Compound Powder, as suspending agent 45, 46
- Mucilage, as suspending agent 45, 47, 48
- Transfusions, aseptic  
ampoule to tube 430-2, 463  
aqueous solutions in sterility tests 462-3  
bottle to bottle 432-435  
Climbic to tube 432  
differences from aseptic distributions 435  
in sterility testing 426, 462  
tube to tube 426-9, 463, 472
- Transfusion equipment, B.S.S. for 296
- Trichlorofluoromethane 517
- Triethanolamine emulsions 65
- Triethylene glycol, aerial bactericide 417
- Triturations 79, 355, 508
- Troschisci (lozenges) 199
- Tryparsamide Injection 248, 277, 455, 464
- Tube  
catgut 467-8  
culture medium 229, 429  
Durham 377, 378, 379, 431  
gas diffusion 395  
needle 340  
ointment 334, 313, 511, 546  
pipette 312, 313  
syringe 539-40
- Tube breaker, catgut 468
- Tube rack 422, 423
- Tuberculin 537, 543
- Tubing  
plastic 285, 286, 369, 519, 520  
rubber 290, 292, 302, 317, 338, 362, 369, 407, 519
- Tubing fluid, catgut 467, 469
- Tubocurarine Injection 276, 277, 358
- Tun dish 333
- Turbidity, bacterial 225, 426, 429, 451, 453, 459, 467  
in Ethanolamine Oleate Injection 394  
in sterility tests 344, 455, 459, 463, 464, 465, 466, 508
- Turpentine emulsion 64
- Tweens 140, 270, 456
- Tyloxapol, in oil sterility tests 466
- Tyndallisation 347-8, 546
- Typical entry in note book 7
- ULTRASONIC cleaning equipment 537, 538
- Unemul 152
- Unguenta, see Ointments 133
- Unit of  
absorbed dose of radiation 531  
radioactivity 530  
ultra violet intensity 528
- Units  
carbon dioxide passage 395, 396  
distribution, see Distribution units  
filtration, see Filtration units  
gas filling (air replacement) 392, 503
- Ultra violet light 224, 415, 526, 527-9  
absorption 260, 527, 528, 531  
air treatment 411, 415, 528-9  
damage to medicaments 283  
hazards 415, 528-9  
effect of humidity on 415 528  
intensity measurement 528  
lamps 404, 415, 527-8  
penetrating power 411, 415, 528, 529
- photochemical damage from 529  
protection from 409, 415, 529  
sterilisation by 309, 415, 527-9
- Unna's paste 149
- Urethral bouge 168
- VACCINE  
B.C.G. 233, 447  
cholera 347  
plague 347  
poliomyelitis 268, 476  
tickettsia 476  
smallpox 233, 473  
sterilisation 346-7, 528, 529, 533  
sterility test 233, 446, 447, 473, 476  
typhoid 347  
viral 292, 473, 476  
yellow fever 268, 473
- Vacuum  
air removal from vials 505  
by steam ejector 330  
desorption of ethylene oxide 520  
expression of degree of 331  
for aseptic distribution 439  
for filling 370  
for seal testing of ampoules 371  
high  
for air removal from sterilisers 331, 336  
for drying dressings 334  
effect on ethylene oxide sterilisation 518
- low  
for air removal 330  
for drying dressings 334  
pre-sterilisation 330-331, 336, 337-8
- Post sterilisation 334, 337, 338, 543
- Pumps, see Pump, vacuum  
supply to asepsis room 404, 405
- Vacuumstat 331
- Valve  
ball, filtration unit 369, 439  
check, steriliser 332, 333  
fine adjustment, gas cylinder 219  
mixing, water 405  
needle, gas 405, 492, 496  
outlet, gas cylinder 218  
reducing  
nitrogen cylinder 405, 485  
steam steriliser 324, 332  
safety, steriliser 325, 327, 520
- Vancomycin hydrochloride 475
- Vapores (inhalations) 181
- Vapourisation, aerial bactericides 417, 418
- gaseous sterilising agents 524
- Vapour, bactericidal 415, 416, 417
- escape line, steam steriliser 332, 333
- pressure, carbon dioxide 517
- Varicose veins, sclerosing agents 249
- Vasopressin Injection 265, 276, 277
- Vehicles  
for aqueous injections 240-44  
alcohol 245  
propylene glycol 245-6  
for oily injections 240, 244-5, 313, 357
- alcohols 244-245  
earachis oil 244-245  
benzyl benzoate 245  
esters 244-245
- Vent  
autoclave 325, 326, 327, 382  
hot air oven 312, 313

Ventilation system, asepsis laboratory 402, 403, 404, 405, 409-21

Venting, of autoclaves 325-8, 331, 337, 543

Venturi, steam steriliser 330, 334

Vial, see Containers

Viomycin 364, 475

Viruses 401, 476, 493, 531

homologous serum, jaundice 310, 529

lethal exposures 310, 316

Viskring, see Seal, plastic ring

Vitamins, instability 265, 277

Volatile substances in

mixtures 42

powders 78

Volume in volume percentages 31

Vulcanisation 290

WALL hatch, asepsis room 403, 405

Walls, asepsis room 403, 404, 408

Washing, facilities for asepsis 402, 405  
476

of hands 424, 430

units 405, 406

Waste disposal, asepsis room 405 406, 422, 423, 424

Water 376

absorption of

  apyrogenic, fresh 243, 312, 362, 364, 365, 376

  aromatic 43

boiling, as sterilising agent 316, 343

ethylene oxide 520

ionising radiations 531

ultra violet light 528

#### Water—(contd)

distilled

  bacterial growth in 223, 241, 364

  for culture media 226

  mould growth in 234

  preparation 242-3

  storage 241, 242

for Injection 240-3, 464, 466, 499

  bactericides in 260

  conductivity 243

  containers 260, 282, 499

  free from Carbon Dioxide 243-4,

  464, 503, 562, 563

  free from Dissolved Air 244,

  267, 276, 563

  preparation 241, 242-3

  pyrogens in, see Pyrogens

  still 242, 332

hot, as sterilising agent 316

Purified 243, 337, 396

seal steam steriliser 332, 333

softeners 362

supply, asepsis laboratory 405

Water, key to 376

Water in-oil emulsions 67, 139

Water miscible ointment bases 160,

161

Wax

Cetomacrogol Emulsifying BPC

56, 141

Cetrimide Emulsifying BPC 56,

144

Emulsifying BP 56, 138

Weathering, see Glass

Weighable quantities 374

Weighing

  aseptic 443, 507

#### Weighing—(contd)

  effect of damp air on 423

  eye ointment ingredients 510

  poisons for injections 365

  small amounts 39

Weights, and measures 9

  standard 11, 14

Weight in volume percentages 17

Weight as weight percentages 28

Wet gum method of emulsification 59

Wettability, of powders 270

Wetting agents 270

White Litment 130

Whiting, rubber filler 289, 292

Windows, asepsis laboratory 403, 405

Witepsol, suppository base 167

Witness tubes 344, 346

Wool alcohol 136, 137, 156, 159

  sterilisation 313

Wool Alcohols Ointment 18, 135, 137

Wool fat 136, 159

  in eye ointment base 509

  sterilisation 313

X RAYS 526, 529, 530, 533

YEASTS 234, 316, 401

Yeast extract 227, 449, 450

ZINC CHLORIDE, in lotion 127

Zinc Gelatin 149, 162

Zinc Ointment 135, 146, 160

Zinc oxide 289, 291, 292, 464

Zinc stearate 289 292

Zinc sulphate 252