

# FUNDAMENTALS OF RADIOBIOLOGY

Z. M. BACQ

*Professor in the University of Liège  
Corresponding Member of the Royal Academy  
of Medicine of Belgium*

and

PETER ALEXANDER

*Chester Beatty Research Institute  
Institute of Cancer Research  
Royal Cancer Hospital  
London*

LONDON

BUTTERWORTHS SCIENTIFIC PUBLICATIONS  
1955

**BUTTERWORTHS PUBLICATIONS LIMITED**  
88 KINGSWAY, LONDON, W.C.2

AFRICA : BUTTERWORTH & CO. (AFRICA) Ltd.  
DURBAN : 33/35 Beach Grove

AUSTRALIA . BUTTERWORTH & CO. (AUSTRALIA) Ltd.  
SYDNEY : 8 O'Connell Street  
MELBOURNE : 430 Bourke Street  
BRISBANE : 240 Queen Street

CANADA . BUTTERWORTH & CO. (CANADA) Ltd.  
TORONTO : 1367 Danforth Avenue

NEW ZEALAND . BUTTERWORTH & CO. (AUSTRALIA) Ltd.  
WELLINGTON : 49/51 Ballance Street  
AUCKLAND : 35 High Street

*U.S.A. Edition published by*  
**ACADEMIC PRESS INC., PUBLISHERS**

125 EAST 23RD STREET  
NEW YORK 10, NEW YORK

*Set in Monotype Baskerville type*  
*Made and printed in Great Britain by William Clowes and Sons, Limited*  
*London and Beccles*

## FOREWORD

The story which the authors of this volume have to tell is a fascinating one. When it is complete it will be the story of how an almost incredibly small amount of energy can change the life of a cell, a tissue, or even an entire organism; but the story is as yet only fragmentary, and the key discoveries lie ahead. Herein lies its fascination to all those who have the good fortune to work in this field.

The number of those engaged in the study of radiobiology is increasing apace, and not entirely, I believe, because of the big practical issues which depend on an extension of knowledge in this field—the improved use of radiation in the treatment of cancer and the avoidance of the hazards to the health of the community which arise from our having entered upon the age of nuclear fission. The subject can offer to a worker in almost any branch of science a definable problem and make almost unlimited demands on his knowledge and technical skill; and biologists in particular may find in radiation a means of disturbing the life of the cell in a controlled manner which may be of value in the study of fundamental processes.

Professor Bacq and Dr Alexander have undertaken the task of presenting a coherent account of the present status of research in this vast field. That the task is one of extraordinary difficulty anyone who has attempted it, on even a limited scale, will testify. This is so not only on account of the amount of material to be reviewed—the 960 original papers quoted in the bibliography represent a year's solid reading—but because of the variety of disciplines to be covered. Multiple authorship is one way of meeting this difficulty but it leaves the reader to build his own bridges. In this volume, Bacq and Alexander, partners in research and experienced teachers, have told us how they see the subject as a whole, and for this we owe them a great debt of gratitude, for the result is a most readable, stimulating and well documented book.

It is particularly valuable at this time to be able to look at radiobiology through the eyes of the physiologist and the chemist, and to compare their presentation with Lea's presentation of certain aspects of the same subject as it appeared to a physicist eight years ago. It is remarkable how few steps have to be retraced. During the intervening years radiation chemistry has been a focus of interest. It is a field in which Dr Alexander's own researches have already

#### FOREWORD

broadened our outlook, and the reader of this book cannot fail to be impressed both by the present vitality of this branch of the subject and by its obvious relevance to radiobiology as a whole.

Professor Bacq's work on the protection afforded by chemical substances, and particularly his extensive studies with cysteamine and cystamine are well known to every radiobiologist. As a physiologist and pharmacologist he has long been specially interested in the reactions of the whole animal to irradiation, and I believe this volume will be welcomed not least for its critical survey of the domains which are at present obscure and tangled—endocrine intervention, the response of mammals to whole-body irradiation, and what the authors call the pathological biochemistry of irradiated tissues. These are domains in which we may confidently expect exciting discoveries in the next decade as we come to grips with the dynamic aspects of radiobiological damage.

L. H. GRAY

British Empire Cancer Campaign Research Unit in Radiobiology,  
Mount Vernon Hospital and The Radium Institute,  
Northwood,  
Middlesex

25 November 1954

## PREFACE

Much of the interest and fascination of the research in radiobiology is that it brings together scientists from many branches. This book is directed to all workers in this field in the hope that it may help to place their own contribution into perspective and to provide a background. Rapid progress can only come from a pooling of the results obtained by physicists, chemists, biologists and clinicians. Each group, immersed in its own problems and with an ever increasing literature appearing in a large number of journals, is finding it difficult to follow relevant developments in adjacent fields. We have not aimed to provide a review for the specialists of individual topics, but have tried to present the subject as a coherent whole. This treatment, will, we hope, also prove of value to radiotherapists, who have for many years used the powerful tool of ionizing radiation successfully in the therapy of cancer, in the absence of an adequate chemical and biological foundation. This position is now being remedied and a less empirical approach to radiotherapy may soon become possible.

This book is a survey and not a monograph. We have selected certain investigations from the enormous mass of published material, and have not attempted to present a complete review of the literature. Also we have deliberately chosen certain aspects of radiobiology for special emphasis since we feel that developments in these fields are most likely to advance the subject. A choice cannot be impartial; but if we have relied to a disproportionate extent on our own researches and on those best known to us we have made every effort to present fully opposing points of view. We have not hesitated to indicate which, in our opinion, are the most acceptable hypotheses at the present time; this has been done to introduce a sense of coherence and does not imply a rigidity of viewpoint and we fully realize that new experimental data may alter the interpretations.

Without the help given to us by colleagues and friends we could not have written this volume and it is a great pleasure to acknowledge our gratitude to them. In particular we should like to thank Professor P. C. Koller, and Dr L. H. Gray whose advice was constantly available to us and who gave us many hours of their time.

The illustrations of chromosomes were prepared for us by Professor Koller, Dr S. H. Revell and Mr L. F. La Cour, and we owe

PREFACE

a great debt to them for the efforts they made to obtain the illustrations we wanted.

The French text was translated by Miss M. Venables, whose helpfulness and patience it is a pleasure to acknowledge.

One of us (P. A.) would like to thank his chief, Professor Alexander Haddow, Director of the Chester Beatty Research Institute, for the encouragement and help he has given at all times to the collaboration between the authors. Z. M. B. is much indebted to the Belgian Government (*Conseil Supérieur de la Sécurité Civile*) for constant material and moral support for more than eight years.

Liège and London,  
October 1954

Zenon M. BACQ  
Peter ALEXANDER

## CONTENTS

	PAGE
<i>Foreword</i>	v
<i>Preface</i>	. vii
1 EFFECTS OF IONIZING RADIATIONS ON MATTER	1
2 DIRECT AND INDIRECT ACTION	46
3 THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS	79
4 EFFECT OF RADIATION ON MACROMOLECULES	121
5 CYTOLOGICAL AND GENETICAL PHENOMENA	156
6 THE NORMAL AND PATHOLOGICAL BIOCHEMISTRY OF MITOSIS	175
7 CHEMICAL SUBSTANCES WHICH SIMULATE THE BIOLOGICAL EFFECTS OF IONIZING RADIATIONS	190
8 THE OXYGEN EFFECT IN RADIobiology	209
9 COMPARATIVE RADIOSensitivity OF LIVING ORGANISMS	220
10 PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS	228
11 PROCESSES OF RESTORATION AFTER IRRADIATION	263
12 INTERVENTION OF THE PITUITARY AND ADRENAL GLANDS 'STRESS' IN RADIATION SICKNESS	267
13 PHYSIOPATHOLOGY AND TREATMENT OF RADIATION SICK- NESS IN MAMMALS	280
14 CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS	290
15 EFFECTS OF PROTECTION OF PART OF THE MAMMALIAN BODY BY A LEAD SCREEN	328
16 INJECTIONS OF HOMOGENATES OF SPLEEN OR BONE- MARROW AFTER IRRADIATION	336
17 OBSERVATIONS IN HUMAN BEINGS	344
POSTSCRIPT	356
NAME INDEX	359
SUBJECT INDEX	369

## ACKNOWLEDGEMENTS

Permission to reproduce illustrations and tabular matter, as follows, is gratefully acknowledged:

Chapter 1, *Figures 3-4*, LEA, D. E., *Actions of Radiations on Living Cells*, Cambridge University Press, 1946; *Figure 5*, HEITLER, W., *The Quantum Theory of Radiation*, Oxford University Press, 1954; *Figures 13-16*, CORMACK, A. and JOHNS, B., *Brit. J. Radiol.*, 1952, **25**, 369; *Figure 18*, TOBIAS, C. A. et al., *Amer. J. Roentgenol.*, 1952, **67**, 1; *Table II*, LEA, D. E., *Actions of Radiations on Living Cells*, Cambridge University Press, 1946.

Chapter 2, *Figure 2*, DALE, W. M. et al., *Phil. Trans.*, 1949, A **242**, 33; *Figure 6* and *Table II*, LEA, D. E., *loc. cit.*, *Figure 7*, TOBIAS, C. A., *Symposium on Radiobiology*, Wiley, New York, 1952, p. 357; *Figure 8*, SETLOW, R. and DOYLE, B., *Arch. Biochem. Biophys.*, 1953, **46**, 46; *Figure 10*, GRAY, L. H., *Brit. J. Radiol.*, 1953, **26**, 609; *Table I*, SETLOW, R. and DOYLE, B., *Arch. Biochem. Biophys.*, 1953, **42**, 83.

Chapter 3, *Table IV*, DAINTON, F. S. and COLLINSON, E., *Annu. Rev. phys. Chem.*, 1951, **2**, 99; *Tables VI-VII*, FRICKE, H. and HART, E. J., *J. chem. Phys.*, 1935, **3**, 60; *Figures 1-2*, BONET-MAURY, P., *Disc. Faraday Soc.*, 1952, **12**, 72; *Figure 3*, DAINTON, F. S. and SUTTON, H. C., *Trans. Faraday Soc.*, 1953, **49**, 1011; *Figure 4*, FRICKE, H. and HART, E. J., *J. chem. Phys.*, 1938, **6**, 229; *Figure 5*, GRAY, L. H., *loc. cit.*; *Figure 6*, LEA, D. E., *Brit. J. Radiol.*, Suppl. No. 1, 1947, p. 59; *Figure 8a*, GRAY, L. H., *Brit. J. Radiol.*, 1953, **26**, 638; *Figure 8b*, ALEXANDER, P. and FOX, M., *Trans. Faraday Soc.*, 1954, **50**, 605; *Figure 9*, FRICKE, H., *J. chem. Phys.*, 1934, **2**, 556; *Figure 10*, EBERT, M. and BOAG, J. W., *Disc. Faraday Soc.*, 1903, **12**, 189; *Figure 11*, FRICKE, H. and BROWNSCOMBE, E. R., *J. Amer. chem. Soc.*, 1933, **55**, 2358, and ALEXANDER, P. and BACQ, Z. M. et al., *Radiation Res.*, 1955 (in the press); *Figure 12*, DALE, W. M. et al., *Biochem. J.*, 1949, **45**, 93; *Figure 13*, DALE, W. M., *Biochem. J.*, 1951, **48**, 129; *Figure 14*, GRAY, L. H., *Brit. J. Radiol.*, 1941, **14**, 102.

Chapter 4, *Tables IV-V*, BARRON, E. S. G., *Symposium on Radiobiology*, Wiley, New York, 1952, p. 216; *Figure 2*, ALEXANDER, P. and FOX, M., *Nature, Lond.*, 1952, **169**, 572; *Figure 3*, ALEXANDER, P. and FOX, M., *J. Chim. phys.*, 1953, **50**, 415; *Figure 5*, BARRON, E. S. G. and FINKELSTEIN, P., *Arch. Biochem. Biophys.*, 1952, **38**, 105; *Figures 8 and 10*, TAYLOR, B. et al., *Arch. Biochem. Biophys.*, 1948, **16**, 19; *Figure 9*, SPARROW, A. H. and ROSENFIELD, F. M., *Science*, 1946, **104**, 245; *Figure 11*, ERRERA, M. C. R., *Bull. Soc. chim. biol.*, 1951, **33**, 555; *Figure 12*, SCHOENBERD, M. D. et al., *U.S. Atom. Energy Comm. U.C.L.A.*, 11, 1949; 83, 1950; *Figure 13*, ERRERA, M. C. R., *Cold Spr. Harb. Symp. quant. Biol.*, 1947, **12**, 60.

Chapter 5, *Figures 7-8, 12, 19*, KOLLER, P. C., *Progr. Biophys.*, 1953, **4**, 195;

## EFFECTS OF IONIZING RADIATIONS ON MATTER

### COMPARISON OF THE DIFFERENT RADIATIONS

IN this book we are concerned with the very short wavelength electromagnetic radiations,  $\text{x}$ - and  $\gamma$ -rays, and the corpuscular radiations made up of electrons ( $\beta$ -rays), helium nuclei ( $\alpha$ -rays), protons and neutrons. The former are radiations of the same character as ultra-violet (u.v.) or visible light, but since they are of much shorter wavelength, the energy of their quanta \* is of the order of  $10^4$  higher than those of u.v. light, so that in practice there is little similarity. The absorption of light waves (infra-red, visible and u.v.) depends in general on the molecular structure of the absorbent and only indirectly on the atomic composition.

The energy of  $\text{x}$ - and  $\gamma$ -rays on the other hand is almost entirely absorbed by ejecting electrons from the atoms through which they pass, and this process is entirely independent of the manner in which these atoms are combined into molecules. Moreover, the amount of energy absorbed from a beam of hard  $\text{x}$ - or  $\gamma$ -rays by a given weight of material is almost independent even of its elementary composition, although this is not so for soft  $\text{x}$ -rays.

It is clear, therefore, that the action of  $\text{x}$ -rays is much less selective than that of light: *e.g.* if u.v. light of  $2600 \text{ \AA}$  is passed through an equal mixture of nucleic acid and a serum protein more than 90 per cent of the energy is taken up by the nucleic acid and less than 10 per cent by the protein. Using  $\gamma$ -rays the same amount of energy would be absorbed by the protein as by the nucleic acid. On absorbing a quantum of light the whole of its energy is stored in the molecule which becomes excited and can then undergo one of a number of different reactions or lose the energy as heat or light (fluorescence).

An atom on absorbing a quantum of  $\text{x}$ - or  $\gamma$ -rays loses an electron. With the exception of extremely soft  $\text{x}$ -rays, with which we are not concerned, the energy of the quantum taken up is greatly in excess

---

\* The energy of each quantum of an electromagnetic radiation in electron volts (eV) is given by  $12,400/\lambda$  (where  $\lambda$  is the wavelength in  $\text{\AA}$ ). The quantum is the smallest step in which radiation can be absorbed, *i.e.* a molecule has to absorb a whole quantum of u.v. light or none at all.

## EFFECTS OF IONIZING RADIATIONS ON MATTER

of that required to produce an ionization (*i.e.* to eject an electron from an atom) and this surplus is stored as kinetic energy in the ejected electron. The latter is then sufficiently energetic to produce ionization in the atoms through which it passes. For the x-rays used in radiobiology almost all the ionizations are produced by the ejected electrons and the effect of initial absorption of the quantum of x-rays is usually neglected. Consequently the ions produced are not distributed at random throughout the solution but are concentrated along the track of the ejected electron. This represents another fundamental difference between u.v. light and ionizing radiation.

If there are no chemical changes all the energy of x-rays as well as of light waves eventually appears as heat in the absorbing material. With the dose rates used in radiobiology a significant change in temperature would not be produced and the heating effect can in general be neglected except perhaps for very densely ionizing radiations or in 'hot spots' where a disproportionate amount of energy is dissipated. In these cases any heating would be accompanied by a high concentration of reactive radicals which would be more damaging than the heat produced.

The distinction between x-rays which are produced in generators and  $\gamma$ -rays which are given off by some radioactive elements has disappeared. Until comparatively recently the most energetic x-rays produced were obtained from 400 kV therapy tubes giving a spectrum ranging in wavelength from 0.03 Å and having an average wavelength of 0.06 Å\*, while  $\gamma$ -rays were obtained from radium with a wavelength of 0.01 Å corresponding to x-rays of  $1.2 \times 10^6$  V. With the development of new machines such as the van de Graaff generator, powerful linear accelerators, betatrons, synchrotrons and microtrons, x-rays corresponding to many million volts can now be generated and these fall within and beyond the wavelength range of  $\gamma$ -rays. The ready availability from atomic piles of the radioactive isotope cobalt-60 ( $^{60}\text{Co}$ ) has provided a useful source of pure  $\gamma$ -rays of high energy, 1.1 to 1.3 MeV†.

$\beta$ -rays—Since the chemical and biological effects of x- and  $\gamma$ -rays are produced by the ejected high-speed electron and not by the primary ionization it follows that similar results can be obtained by direct bombardment with electrons of comparable energies. Such

---

\* In the spectrum of x-rays given out by therapy-type machines the most energetic radiations (*i.e.* those of shortest wavelength) have an energy equivalent to the peak voltage [*i.e.* their wavelength is  $\lambda = 12.4/(\text{kV of set})$ ]. However, the average energy of all radiations is according to LEA<sup>1</sup> half this value.

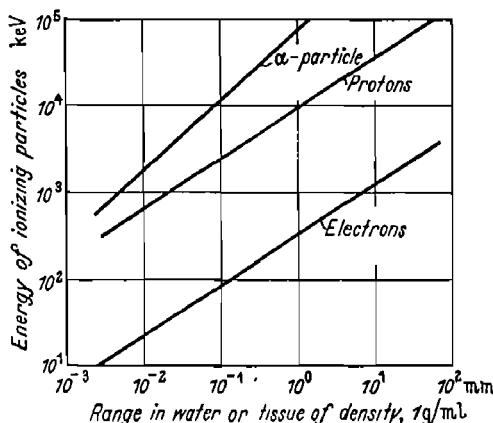
† The electron volt (eV) is a unit of energy corresponding to  $1.60 \times 10^{-12}$  ergs. 1 MeV =  $10^6$  eV, 1 keV =  $10^3$  eV.

## COMPARISON OF THE DIFFERENT RADIATIONS

electron beams are called  $\beta$ -rays and can either be obtained from special generators or from radioactive isotopes of which a large choice is now available (see *Table I*). The distance of penetration of  $\beta$ -rays depends on their energy (see *Figure 1*), but even with 2 MeV electrons the range in water (or in biological tissue) is only 5–7 mm. However the disadvantage of the short range of the  $\beta$ -rays can be overcome by dissolving radioisotopes in the solution or system which is to be irradiated when the whole volume will be uniformly exposed. In biological systems the isotope may become localized in certain regions and the resultant irradiation will then not be uniform.

*Heavy ionizing particles*— $\alpha$ -rays are the nuclei of helium atoms (*i.e.* double charged positive particles of atomic weight 4). They are given off by a few radioactive substances, notably radon—obtained

*Figure 1. Relationship between the range of an ionizing particle in water (mm) and its energy (keV)*



as a decay product from radium—and polonium\*. The latter is a pure  $\alpha$ -emitter while radon also gives off  $\beta$ -rays. Because of their high charge and low velocities the particles are readily stopped by matter and in water or tissue the range of a particle from radium C<sup>1</sup> is only 7.0  $\mu$  (see *Figure 1*), and many ions are formed along its track (*i.e.* the ionization density is very high, see p. 20).

Protons are hydrogen nuclei having mass 1 and carrying one charge; they can be obtained artificially from the cyclotron, proton-synchrotron or a van de Graaff generator. Their properties such as ion density and penetration are intermediate between those of the  $\alpha$ -particle (mass 4) and the electrons (mass  $5.5 \times 10^{-4}$ ).

---

\*  $\alpha$ -rays of very low energy and consequently giving an extremely high ion density can be obtained by the artificial disintegration of boron or lithium by slow neutrons. For example when the nucleus of a lithium atom captures a neutron it immediately dissociates to give an  $\alpha$ -particle. Tritium ( ${}^3\text{H}$ ) remains and this decays slowly by giving off  $\beta$ -rays.

## EFFECTS OF IONIZING RADIATIONS ON MATTER

 Table I. List of Some  $\beta$ -ray Emitting Isotopes

Element	Z	A	Half-life, hour, day or year	Radia- tion	%	$E_{\max}$ MeV	Formation in the pile
H	1	3	11.8 y	$\beta^-$	—	0.018	$^2H(n\gamma)^3H$ , $Li(n\alpha)^3H$
Be	4	10	$2.5 \times 10^6$ y	$\beta^-$	—	0.555	$^9Be(n\gamma)^{10}Be$
C	6	14	5568 y	$\beta^-$	—	0.155	$^{13}C(n\gamma)^{14}C$
Na	11	22	2.7 y	$\beta^+$	—	0.557	—
P	15	32	14.3 d	$\beta^-$	—	1.701	$^{31}P(n\gamma)^{32}P$
	15	33	25 d	$\beta^-$	—	0.26	—
S	16	35	88 d	$\beta^-$	—	0.167	$^{34}S(n\gamma)^{35}S$
Cl	17	36	$4 \times 10^5$ y	$\beta^-$	—	0.714	$^{35}Cl(n\gamma)^{36}Cl$
K	19	40	$1.3 \times 10^9$ y	$\beta^-$	89	1.33	Naturally occurring
			$1.3 \times 10^9$ y	$\gamma$	11	1.46	—
Ca	20	45	152 d	$\beta^-$	—	0.255	$^{44}Ca(n\gamma)^{45}Ca$
				$\beta^-$	—	0.46	$^{58}Fe(n\gamma)^{59}Fe$
Fe	26	59	47 d	$\beta^-$	50	1.1	—
				$\beta^-$	50	0.26	—
				$\beta^-$	50	1.30	—
As	33	77	40 h	$\beta^-$	—	0.80	$^{76}Ge(n\gamma)^{77}Ge \xrightarrow{\beta^-} ^{77}As$
Br	35	82	34 h	$\beta^-$	—	0.447	$^{81}Br(n\gamma)^{82}Br$
					—	0.323	—
					—	0.181	—
—	—	—	—	$\gamma$	16	1.321	—
					18	1.036	—
					65	0.769 etc.	—
Rb	37	86	19.5 d	$\beta^-$	80	1.822	$^{85}Rb(n\gamma)^{86}Rb$
				$\beta^-$	20	0.716	—
				$\gamma$	—	1.081	—
Sr	38	89	53 d	$\beta^-$	—	1.463	$^{88}Sr(n\gamma)^{89}Sr$
Ag	47	110	270 d	$\beta^-$	58	0.087	$^{109}Ag(n\gamma)^{110}Ag$
					35	0.570	—
					5	2.90	—
—	—	—	—	$\gamma$	—	1.48	—
					—	0.9	—
					—	0.66 etc.	—
Ag	47	111	7.5 d	$\beta^-$	91	1.04	$^{110}Pd(n\gamma)^{111}Pd \xrightarrow{\beta^-} ^{111}Ag$
				$\beta^-$	—	0.70	—
				$\gamma$	8	0.34	—
				$\beta^-$	—	0.80	—
I	53	131	8 d	$\beta^-$	1	0.24	—
				$\gamma$	86	0.605	—
				$\beta^-$	—	0.364 etc.	$^{130}Te(n\gamma)^{131}Te \xrightarrow{\beta^-} ^{131}I$
				$\gamma$	—	—	—
				$\beta^-$	14	0.25	—
				$\gamma$	—	0.637	—
Au	79	198	2.69 d	$\beta^-$	—	0.96	$^{197}Au(n\gamma)^{198}Au$
				$\gamma$	—	0.441	—
Hg	80	203	43.5 d	$\beta^-$	—	0.208	$^{202}Hg(n\gamma)^{203}Hg$
				$\gamma$	—	0.279	—
Tl	81	204	2.7 y	$\beta^-$	—	0.775	$^{203}Tl(n\gamma)^{204}Tl$
RaE(Bi)	83	210	5.02 d	$\beta^-$	—	1.17	Naturally occurring

Z is the atomic number; A is the atomic weight.

#### COMPARISON OF THE DIFFERENT RADIATIONS

With the newer generators many heavy ionizing particles can now be produced. Any atom stripped of one or more of its electrons if accelerated will become an ionizing particle. Deuterons are frequently used; they have mass 2, charge 1 and consequently their penetration and ionization density is intermediate between that of protons and  $\alpha$ -particles. Carbon atoms which have lost six electrons,  $C^{6+}$ , are probably the most densely ionizing particles used in radiobiology. With a mass of 12 and charge of 6 they are almost as different from  $\alpha$ -particles as electrons are from protons.

*Neutrons*—Fast neutrons (particles having mass of 1 but carrying no charge) are usually obtained either from a cyclotron, atomic pile or indirectly from a van de Graaff generator, but can also be obtained more simply by the bombardment of beryllium with  $\alpha$ -particles. A simple low-power source is the complex salt  $RaBeF_4$ . Neutrons do not produce ionization directly but knock out protons from the nucleus of the atom they traverse. The biological effects of fast neutrons are, therefore, almost wholly due to protons in exactly the same way as the effects of x-rays are produced by the ejected electrons. Unlike the other ionizing radiations, however, the number of ionizations produced depends largely on the nature of the elementary composition of the material through which the neutrons pass. The reason for this is that the transfer of energy between neutrons and protons does not depend on the atomic number but on other factors, and the number of ionizations produced by a given dose of neutrons in 1 g of water will be about 2·5 times that produced in 1 g of air; this makes neutron dosimetry very difficult (see p. 15). Neutrons, like x-rays, can penetrate large amounts of matter since the protons are ejected at random within the irradiated material. The ionizations are, therefore, concentrated along short tracks inside the irradiated body.

Slow neutrons do not eject a proton but are captured by the nuclei through which they pass, thereby producing a new nucleus which is radioactive and will emit  $\beta$ - or  $\gamma$ -rays. During the process of neutron capture the nucleus emits a  $\gamma$ -ray. Many of the radioactive substances listed in *Table I* are produced in this way in atomic piles. The reactions of slow neutrons, although of much chemical interest, are unlikely to be of great biological importance since the effects produced by the ionizing radiations emitted are much more far-reaching than those resulting from the transmutation of relatively few atoms. In this connection it should be pointed out that very high energy electromagnetic or  $\beta$ -radiations (*i.e.* greater than 8 MeV), produced for example by a synchrotron or betatron, will also produce nuclear transformations in some of the elements

## EFFECTS OF IONIZING RADIATIONS ON MATTER

through which they pass. An animal irradiated from one of these generators becomes detectably radioactive. In x-ray therapy with a 25 MeV betatron 5 per cent of the total dose received by the patient is emitted by the carbon isotope  $^{11}\text{C}$  which is produced *in situ* from the ordinary  $^{12}\text{C}$  atoms in the body by the x-rays. A case is recorded where the gold tooth of a man accidentally exposed to slow neutrons became so radioactive as to produce ulceration of the gum.

## MECHANISM OF ENERGY LOSS BY IONIZING RADIATIONS

Interactions between beams of electromagnetic or particulate radiation and matter can only be described quantitatively in the language of quantum mechanics. The problem, although very difficult, has been solved by contemporary physics and detailed treatments are given in advanced modern textbooks<sup>2</sup>. It is not possible here to do more than give a list of some of the more important processes. Excitation of atoms and molecules by the absorption of a quantum of visible or u.v. light will not be considered.

As we have seen, virtually all the ionizations which result from the absorption of x- or  $\gamma$ -rays are produced by the ejected electrons. The first problem is, therefore, to determine the number and energy of the electrons produced when these rays are absorbed. For all radiations the energy (or intensity) of the beam before absorption ( $I_0$ ) is related to that after absorption ( $I$ ) by the equation  $I = I_0 e^{-\mu x}$  where  $\mu$  is the absorption coefficient and  $x$  the amount of material. The thickness  $x$  may be expressed variously as cm, g/cm<sup>2</sup>, atoms/cm<sup>2</sup>, or electrons/cm<sup>2</sup>. Since the product  $\mu x$  must be dimensionless,  $\mu$  is correspondingly expressed as cm<sup>-1</sup>, cm<sup>2</sup>/g, cm<sup>2</sup>/atom, or cm<sup>2</sup>/electron. To indicate which unit is being used the following symbols are conventionally employed :

$$\begin{aligned} \mu_e &\text{ for cm}^2/\text{electron}, & \mu/\rho &\text{ for cm}^2/\text{g (mass coefficient),} \\ \mu_a &\text{ for cm}^2/\text{atom}, & \mu &\text{ for cm}^{-1}. \end{aligned}$$

All these coefficients can be interconverted if the atomic weight ( $A$ ) and the atomic number ( $Z$ ) are known ; e.g. in terms of  $\mu_e$ ,

$$\begin{aligned} \mu_a &= Z\mu_e \\ \mu/\rho &= N(Z/A)\mu_e \\ \mu &= \rho N(Z/A)\mu_e \end{aligned}$$

where  $N$  is Avogadro's number and  $\rho$  the density.

There are essentially three mechanisms by which energy can be transferred from the radiations to the material through which they pass and, when scattering can be neglected as is normally the case,

## MECHANISM OF ENERGY LOSS BY IONIZING RADIATIONS

$\mu_a$  is made up of three components,  $\tau_a$ ,  $\sigma_a$  and  $\pi_a$ , corresponding to energy absorption by the photoelectric effect, Compton effect and pair formation.

*The photoelectric effect*—By this mechanism a quantum gives up all its energy (*i.e.* is completely absorbed) to an atomically bound electron which it ejects. The kinetic energy of this electron is the energy of the quantum less the energy required to remove the electron from the atom (the binding energy). Since electrons at different levels have different binding energies the energy of the photo-electron will vary, but for the atoms making up organic materials and water a maximum value for the binding energy of 500 eV may be taken\*. Compared with the high quantum energy of the radiations used in radiobiology the binding energy is comparatively so small that virtually all the energy is retained by the photoelectron which then produces further ionizations.

The absorption coefficient per atom  $\mu_a$  of the material varies with the wavelength,  $\lambda$ , of the radiation and the atomic number,  $Z$ , of the elements of which it is composed. The atomic absorption coefficient for photoelectric absorption ( $\tau_a$ ) is given by

$$\tau_a = c \cdot \lambda^m \cdot Z^n$$

where  $c$  is a constant,  $m$  is of the order of 3·5 to 5. Consequently the photoelectric absorption falls off very rapidly as the radiations become more energetic (*i.e.* harder), and for x-rays of energy greater than 1 MeV the contribution of photoelectrons to the total energy absorption can be neglected (see *Figure 2*). Also since the absorption varies as a high power of  $Z$  the photoelectric absorption is much greater for heavy elements than for light elements.

*The Compton effect*—The elementary view is that this process is a ‘billiards ball’ like collision between the quanta of radiation† and the outer shell electrons of the atoms through which they pass. The amount of energy transferred to the electron which is ejected varies and can be calculated from the theoretically derived equation

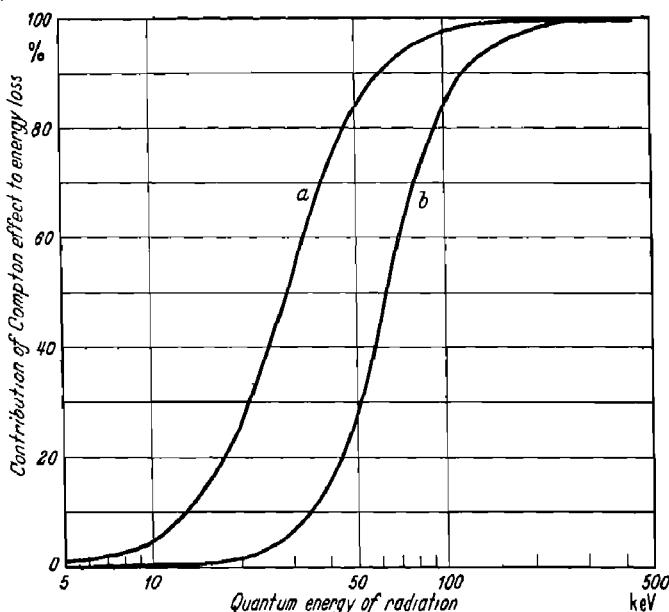
\* When an inner electron has been ejected an outer or free electron can fall into its place. In this process energy is set free since the gross change is the removal of an outer electron which requires only about 10 eV compared with the value of about 500 eV for inner electrons. This energy is liberated as a quantum of radiation—corresponding to very soft x-rays—which is usually absorbed by the same atom to give an electron of extremely low energy having a high specific ionization (see p. 19). This gives rise to a highly localized release of about 500 eV and is referred to as the Auger effect.

† The scattered quantum after it has given up a fraction of its energy to the ejected electron will behave normally and can undergo all the processes for energy loss (*e.g.* another ‘Compton collision’).

## EFFECTS OF IONIZING RADIATIONS ON MATTER

given by KLEIN and NISHINA<sup>3</sup>. The energy of these recoil electrons, unlike that of the photoelectrons, varies widely and ranges from zero to a maximum value. The average energy of the recoil electrons increases rapidly with the energy of the radiation as shown in *Figure 3*. Consequently the contribution of Compton electrons to the total amount of energy absorption increases with the hardness of the radiation (see *Figure 2*), although the overall absorption coefficient decreases with decreasing wavelength (see *Figure 5*).

Besides the difference in the energy of the electrons ejected by the



*Figure 2. Relative importance of Compton and photoelectric effect for energy loss in water by x-rays of different quantum energy: (a) proportion of total number of electrons produced by Compton effect; (b) proportion of total energy which appears in recoil (Compton) electrons. (The difference between the value shown and 100 per cent is due to photoelectrons; in the range of energies shown, pair formation (see p. 10) does not occur.)*

photoelectric and Compton effects there is an important difference in the energy dissipation in different materials. The contribution of the Compton effect to the mass absorption coefficient of the material under irradiation (*i.e.* the amount of energy dissipated per gramme) depends entirely on the number of electrons per gramme, and this in turn depends on the elementary composition (see *Table II*). Fortunately this value does not vary greatly for different elements and is nearly the same for water, most organic materials and consequently biological tissue. This means that if the source

#### MECHANISM OF ENERGY LOSS BY IONIZING RADIATIONS

of radiation of hard x-rays where the Compton effect predominates has been calibrated by measuring energy dissipation per gramme of air with a standard ionization gauge this value can be converted by

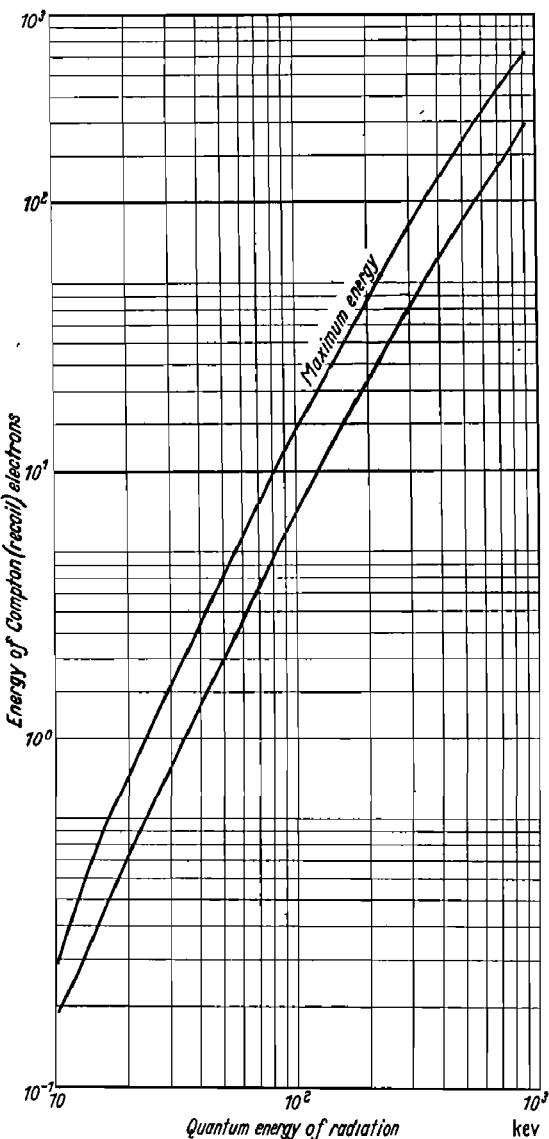


Figure 3. Maximum and mean energy of Compton (recoil) electrons as a function of quantum energy of radiation in eV. (The average quantum energy of the radiations from an x-ray therapy set is half the peak voltage.) (Data taken from LEA<sup>1</sup>.)

a common factor (see *Figure 4*) to give the energy loss in all biological materials.

The position is more complex for soft x-rays where a considerable proportion of the energy dissipation is due to the photoelectric effect

## EFFECTS OF IONIZING RADIATIONS ON MATTER

 Table II. (After LEA<sup>1</sup>)

Element	Atomic number	No. of electrons per gramme $\times 10^{-23}$
H	1	5.98
C	6	3.01
N	7	3.01
O	8	3.01
Mg	12	2.97
Al	13	2.90
P	15	2.91
S	16	3.01
Cl	17	2.89
Ca	20	3.01
Air	—	3.01
Water	—	3.34
Nucleoprotein*	—	3.21
Wet tissue*	—	3.30

\* For definition see Figure 4.

since the absorption of a material is no longer directly proportional to the number of electrons. The energy dissipation is not now essentially the same for water tissue and protein and the values relative to air are given in Figure 4 for x-rays of different wavelengths. The important point is that for soft x-rays it is not possible to derive the dose for different systems directly from measurements with an ionization chamber. Corrections have to be applied which depend upon the elementary composition of the irradiated material. These corrections are not easy to make accurately when the x-rays are not monochromatic, and for 20 kV x-rays the difference in energy dissipation for the same exposure between 1 g of protein and 1 g of water is 15 per cent. For this reason chemical dosimetry methods, especially those using organic solvents, are suitable only for hard x-rays and liable to serious error when used for calibrating therapy sets working at 150 kV or less.

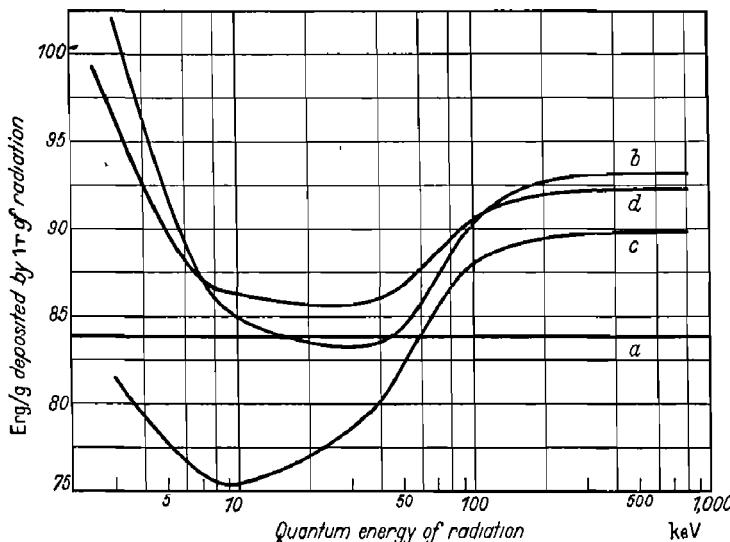
*Electron-positron production*\*—Radiations with an energy greater than 1.02 MeV can lose energy by the simultaneous ‘creation’ of an electron and a positron. The effect is complex and can be understood only in terms of quantum and wave-mechanics. Certain points, however, may be noted: all the energy of the quantum appears in the pair of particles, the first 1.02 MeV providing the ‘rest-mass’ and the remainder providing the kinetic energy of the particles. The two particles then lose their energy by collisions

\* This phenomenon is usually referred to as pair formation and this term must not be confused with ‘ion pairs’.

## MECHANISM OF ENERGY LOSS BY IONIZING RADIATIONS

with electrons, but in addition the positron has the possibility, the greater the lower the kinetic energy, of annihilation with an electron. In this event, which all positrons eventually undergo, the mass of the two particles is lost and appears as energy in two quanta of **x**-rays. In the region of biological interest the atomic absorption coefficient for pair formation varies as  $Z^2$  and is, therefore, greater for 1 g of a heavy element than 1 g of a light element.

The mass absorption coefficient of air and water after passing through an inflection (see p. 26, *Figure 14*) for radiations of



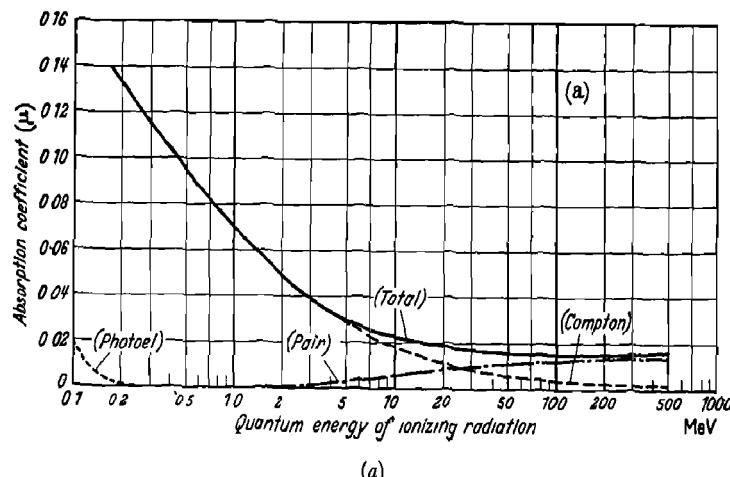
*Figure 4.* Relationship between the amount of energy absorbed by media of different compositions for 1 r of various radiations (after LEA<sup>1</sup>). Curve (a) air (this is fixed by the definition of the roentgen); (b) water; (c) dry nucleo-protein (assumed elementary composition H 7, C 49, N 16, O 25, P 1, S 0.5, ash 1.5 per cent); (d) wet tissue (assumed elementary composition H 10, C 12, N 4, O 73, Na 0.1, Mg 0.04, P 0.2, S 0.2, Cl 0.1, K 0.35, Ca 0.01 per cent).

about 100 keV decreases with increasing energy of the radiation since energy loss by both the Compton and photoelectric effect decreases. At high energies the absorption increases again because energy loss by electron-positron formation increases with increasing energy of the radiation. This complex behaviour is illustrated in *Figure 5* for elementary carbon and water. The minimum depends upon the elementary composition of the irradiated material and lies at lower energies for heavier elements.

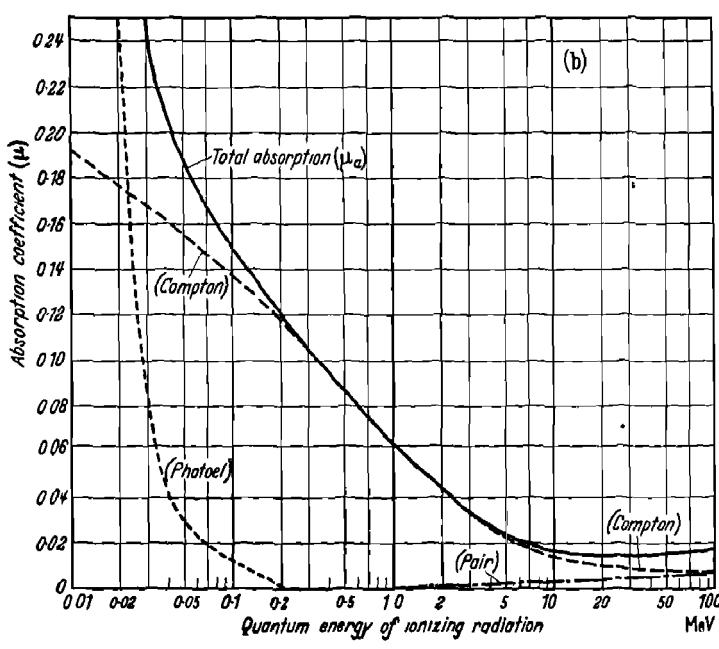
**Cerenkov radiation**—In media such as water for which the optical index of refraction ( $n$ ) is greater than one, the velocity of light is  $c/n$  where  $c$  is the velocity *in vacuo*. A charged particle can never

## EFFECTS OF IONIZING RADIATIONS ON MATTER

move faster than  $c$ , but high energy electrons can move faster than  $c/n$ . A particle moving through a medium at a speed greater than  $c/n$  has its electric field strongly perturbed and loses some of its



(a)



(b)

*Figure 5. Relative contribution of photoelectric effect, Compton effect and pair formation to the mass absorption coefficient of ionizing radiations having different quantum energies (after HEITLER<sup>2</sup>): (a) for water; (b) for carbon. (The minimum at about 100 keV cannot be seen on the scale of this diagram but is clearly shown in Figure 14.)*

#### MECHANISM OF ENERGY LOSS BY IONIZING RADIATIONS

energy—usually only a very small part—as radiation which is named after its discoverer Cerenkov. This radiation is often in the u.v. or visible region and is unlikely to have any biological significance<sup>5</sup>.

*Energy loss by particulate radiations*—Although the fundamental particles differ from one another in size and charge their mechanism of energy loss is essentially the same. The only exception is the neutron which, of course, cannot participate in processes depending on electric charge, it however produces protons and  $\gamma$ -rays which lose energy in the normal ways.

The charged particles undergo inelastic collisions with the bound electrons of atoms which they eject to produce ions. Some of these ejected electrons may be sufficiently energetic to produce ionizations of their own. The energy of all these particles is used up in removing the bonded electron (*i.e.* in ionization) and in producing excited atoms until all the electrons have become of such low energy that they can no longer produce ionizations and are captured to form negative ions (see p. 22). A formula of the loss of energy by  $\alpha$ -particles was first derived by Niels Bohr and extended by BETHE<sup>4</sup> to cover the case of electrons where special quantum effects must be allowed for. The absorption of energy per grammme of material depends, as does the Compton effect, on the number of electrons present and consequently the stopping power\* is essentially the same for water tissue and most organic materials. It may be mentioned again that these processes are responsible for virtually all the energy taken up from x- or  $\gamma$ -rays and that the photoelectric effect, Compton effect and pair formation merely determine the number and energy of the electrons which produce the ionizations. Essentially there is no difference between the effects produced by fast electrons and by x-rays except that the range of the former is small while the latter can penetrate much deeper and release electrons in the interior.

It must be stressed here that all these considerations apply only to the initial act of ionization. In mixed systems, particularly mixtures of gases, transfer of ionization can take place to the most easily ionized molecules so that the relative proportion of molecule finally ionized need bear no direct relation to their stopping power.

#### UNITS OF RADIATION DOSE AND OF RADIOACTIVITY

The most definite quantity which can be measured when matter is exposed to ionizing radiations is the amount of energy gained. On

---

\* For particulate radiations the term 'stopping power' is used; it is defined in exactly the same way as the absorption coefficient of electromagnetic radiation.

## EFFECTS OF IONIZING RADIATIONS ON MATTER

bombardment with particulate radiation (*e.g.*  $\alpha$ - or  $\beta$ -rays) these particles are also retained by the absorbing substance, but with the exception of slow neutrons this effect can be neglected for the purpose of radiobiology.

The energy taken up manifests itself in different forms and eventually appears as heat and chemical change. A very small amount, which need not be considered may be given off as light. Ideally, therefore, radiation dose should be measured as the amount of heat produced in a system which does not undergo any net chemical change. However, the amount of energy involved in radiobiology and radiation therapy is much too small to be detected calorimetrically and there are few radiation sources for which this is possible (see p. 19). For example, a dose of x-rays sufficient to kill a mammal (*i.e.* 700 r) would raise its temperature only by  $1.7 \times 10^{-3}$  °C even if all the energy were converted to heat and none in chemical change.

*The roentgen*—In gases the most readily detected manifestation of energy absorption is the formation of an ion pair. Although, as will be shown later, all the energy absorbed is not utilized in producing ionization and some of it produces excitations (see p. 30) the amount of energy which has to be provided by ionizing radiations to produce one ion pair together with its associated excitations is known experimentally and referred to as  $W$ , the energy required to produce an ion pair. For this reason the dose of x- and  $\gamma$ -rays is usually defined in roentgens (r). One r (see *Table III*) is defined as that quantity of x- or  $\gamma$ -radiation such that the associated corpuscular emission (*i.e.* electrons) per 0.001293\* of air produce in air ions carry 1 e.s.u. of electricity of either sign. From classical electrochemistry (Faraday's Law) 1 e.s.u. is known to correspond to  $2.1 \times 10^9$  ion pairs and consequently this is the number of ion pairs formed in 1 cc of air by 1 r. Since  $W = 32.5$  eV ( $= 5.2 \times 10^{-11}$  ergs) 1 r represents an energy absorption of 0.107 ergs per cc of irradiated air or 83 ergs per gramme of air or 93 ergs per gramme of water or tissue. The mass stopping power of water vapour is greater than that of air because it differs in atomic composition. Therefore, the number of ion pairs formed by 1 r in water vapour is greater than that formed in air although the values for  $W$  are similar. For the purpose of radiochemical calculation (see p. 36) the values are assumed to be the same, but this is arbitrary. It must be stressed that all this applies to gases only, since there is no direct evidence concerning the number of ion pairs formed in liquids or solids (see p. 24).

---

\* 0.001293 g of air occupy 1 cc at N.T.P. (*i.e.* 0° C and 760 mm).

## UNITS OF RADIATION DOSE AND OF RADIOACTIVITY

*Table III. Radiation Units*

1 roentgen (r)	<i>produces in 1 cc of air at N.T.P.* 1 e.s.u. of electricity of either charge</i>
1 , ,	<i>produces <math>2.1 \times 10^9</math> ion pairs per cc of air</i>
<i>Exposure of air to 1 r results in an absorption of 83 ergs/g</i>	
<i>Exposure of water (or tissue) to 1 r results in an absorption of 93 ergs/g</i>	
1 roentgen equivalent physical (rep)†	<i>releases the same amount of energy in water (or tissues) as 1 r of x-rays</i>
1 v unit	<i>dose of neutrons which produces in water an amount of corpuscular radiation corresponding to 1 rep</i>
n unit‡	<i>dose of neutrons which produces in a 100 r 'Victoreen' ionization chamber the same amount of ionization as 1 r of x-rays</i>
1 rad§	<i>corresponds to an absorption of 100 ergs/g</i>
<i>Energy to form an ion pair in air (W)</i>	<i>For x- and <math>\gamma</math>-rays 32.5 eV. For heavy particles (e.g. protons and <math>\alpha</math>) 35 eV</i>
1 curie	<i>The amount of radioactive material which will give <math>3.7 \times 10^{10}</math> disintegrations per sec</i>
K factor	<i>gives the dose in r per hour at 1 cm distance in air of <math>\gamma</math>-rays given off by 1 milliecurie (<math>10^{-3}</math> curies) of a radioactive material. The value of this constant depends on the energy of the emitted <math>\gamma</math>-rays</i>

\* 1 cc of air at N.T.P. weighs 0.001293 g

† Sometimes the rep 83 is used which means that the radiation and (e.g.  $\alpha$ ,  $\beta$  etc.) releases the same energy as 1 r of x-rays in air. Since the ratio of the absorption coefficients may vary (in particular for neutrons) the two rep units need not be the same.

‡ The n unit is unsatisfactory as it depends on the instrument used. One n is believed to correspond to 2.5 v units.

§ For x-rays 1 rad = amount of energy released by 1.08 r in water.

The roentgen has the great merit, after earlier definitions, of being unambiguous, but its measurement is often a matter of considerable difficulty. Confusion also has arisen by attempts to extend the definition of the roentgen to radiations other than x- and  $\gamma$ -rays.

The rad—Since the roentgen is defined for x- and  $\gamma$ -rays an extension was made to cover other radiations. The rep (roentgen equivalent, physical) is defined as that quantity of radiation which will release in water (or tissue) the same amount of energy as is released by 1 r of x-rays. Unfortunately the ambiguity remains whether this means 83 ergs per gramme of air or 93 ergs per gramme of water. In principle the extension of a unit based on measuring

## EFFECTS OF IONIZING RADIATIONS ON MATTER

ion pairs is not suitable for energy absorption in condensed systems. To avoid these difficulties it has been proposed by the international committee<sup>6</sup> to define dosage by an unambiguous energy unit ( $E_m$ ).<sup>\*</sup> The name rad is suggested for the quantity of radiation which will result in an energy absorption of 100 ergs by the irradiated material. It should be recalled that MAYNEORD<sup>7</sup> introduced in 1940 an energy unit which has been widely used in radiotherapy known as the gramme roentgen. By a direct transfer of quantities 1 gramme roentgen is defined as 83 ergs of energy dissipated in the irradiated material.

The rad covers all radiations, including that of neutrons, which are detected by the ionizations produced by the protons they cause to be emitted. The existing units for neutrons are not very satisfactory<sup>8</sup>. Since the number of protons present in 1 gramme of water is very much greater than that in 1 gramme of air, the ratio of the energy absorbed in the two media may be as much as 10 for neutrons as compared to 1.12 for the other radiations.

*Activity of radioactive materials*—Samples of radioactive isotopes are calibrated both in terms of the activity of the sample and the energy of the radiation (see *Table I*). For clinical use and in radiobiological experiments  $\beta$ -emitters are usually used, although in many cases  $\gamma$ -rays are also given off and will contribute to the total dose received by a volume of tissue. The generally accepted unit of activity is the curie, which was originally defined as that quantity of radon or other disintegration product of radium which is in equilibrium with 1 g of radium. This has been modified as the amount of material in which  $3.70 \times 10^{10}$  atoms disintegrate per second. If we know the energy of the radiations given off by the disintegrating atoms it is possible to calculate the radiation dose received. For example, one curie of an isotope giving off  $\beta$ -radiations of average energy 1 MeV when dissolved in 1 litre of water (or dispersed in 1 litre of tissue) will result in an absorption of  $3.7 \times 10^{10} \times 1 \times 10^6 \times 10^{-3}$  eV/g/sec = 0.6 rad/sec and the dose rate will be 0.64 rep/sec. For the purpose of this calculation it is assumed that the energy of the  $\beta$ -rays is wholly dissipated in the dissolved volume.<sup>†</sup> If the volume in which the radioactive material is suspended is small, this assumption will no longer apply, since the

\*  $E_m = WS\mathcal{J}_m$ ;  $E_m$  = energy imparted to unit mass of substance;  $\mathcal{J}_m$  = number of ionizations in unit mass of gas;  $W$  = energy per ion pair of gas;  $S$  = ratio of mass stopping power of the material to the gas.

† The following formula<sup>61</sup> gives the dose (D) by water or tissue in rep per day when it can be assumed that all the energy is dissipated in the volume under investigation.  $D = 53 CE$ , where  $C$  is the concentration of the isotope in microcuries/g, and  $E$  the energy of the radiation in MeV.

## UNITS OF RADIATION DOSE AND OF RADIOACTIVITY

range of the radiations may then extend beyond the volume studied. The activity, and therefore the dose rate, will of course decrease with time. The half-life of an isotope is the period in which its activity falls to one half.

Since the range of  $\gamma$ -radiations is so very much greater it is necessary to treat these differently from  $\beta$ -rays, and the  $K$  constants calculated by MAYNEORD<sup>9</sup> are generally used. This very important field of calibrating radioactive isotopes is dealt with in an excellent review by SINCLAIR and LAMERTON<sup>10</sup>.

## MEASUREMENT OF DOSE

*Physical dosimetry*—The absolute calibration of x- or  $\gamma$ -rays is made by measuring the saturation current in air in a parallel plate ionization chamber. The general principle is as follows: a closed chamber provided with electrodes is subjected to a steady beam of ionizing radiation which will produce a fixed number of ions per second. The electrodes are connected across a variable source of d.c. voltage. As the applied voltage is increased the current at first increases proportionally (*i.e.* the ionization chamber obeys Ohm's law). At higher voltage the current becomes constant since all the available ions are being attracted to the electrodes and a further increase of applied voltage cannot increase the amount of charge carried. From this saturation current the number of ion pairs formed by the radiation to which the chamber is exposed can be directly calculated. No direct determination is possible for the number of ion pairs formed in liquids or solids since a saturation current cannot be obtained even at the highest voltages.

According to the definition of the roentgen all the ions associated with 1 cc of air have to be collected by the electrodes. Associated ions are those which are produced by electrons originating in the defined volume. Consequently the parallel plate chamber must have dimensions which are at least twice those of the range of the electrons. For 5 MeV x-rays this would necessitate a length of 40 metres and is clearly impracticable. This difficulty is avoided by irradiating a solid material and measuring the saturation current in a cavity within it. GRAY<sup>11</sup> developed a theory of Bragg to relate the dose received by the solid—in which the path of the electrons is, of course, about one thousand times shorter—with the number of ionizations produced within the cavity. This value depends on the atomic composition of the solid (usually a plastic) and the nature of the gas.

The routine instrument is the 'Victoreen' dosimeter, which

## EFFECTS OF IONIZING RADIATIONS ON MATTER

consists of a thimble-shaped condenser having a thin wall of plastic material with mass stopping power equivalent to air. This condenser is initially charged and the amount of discharge due to ionization is a measure of the dose and can be read directly on a scale. A similar instrument can be used to measure the dose from a beam of electrons ( $\beta$ -particles) but usually rather large corrections have to be applied.

The dose from  $\alpha$ -particles has generally to be computed from the total number of  $\alpha$ -particles involved—determined photographically or with a counter—and the energy dissipated by each particle.

*Energy (W) required to form an ion pair in air*—The physical methods measure the number of ion pairs produced, while the value required for the dose is an energy unit. It is, therefore, necessary to know the energy needed to form an ion pair in air (referred to as  $W$ ). After careful consideration of all the available data GRAY<sup>12</sup> proposed that  $W = 32.5$  eV for x-,  $\gamma$ - and  $\beta$ -rays, and 35 eV for  $\alpha$ -rays, protons and other heavy particles. These values are independent of the pressure of the gas; the same values are usually also adopted for the energy needed to form an ion pair in water.

$W$  can be determined by essentially four different methods, all of which give the same value within experimental error. The values put forward by Gray are average values, judiciously weighted according to the method used, and almost certainly correct to within two or three per cent. If we make the assumption (see p. 24) that  $W$  for liquids is the same as for gases, then the concentration of ion pairs produced by 1 r in water is of the order of  $3 \times 10^{-9} M$ .

*Chemical dosimetry*—In practice the use of an air-filled ionization chamber is difficult for hard radiations and many attempts have been made to develop a chemical method. In principle any chemical reaction, depending upon the dose of ionizing radiation and which can be easily and accurately measured, could be used as an integral dosimeter. However, for such a method to be useful the reaction must be (1) independent of dose rate over a wide range; (2) independent of the ion density over a wide range; (3) carried out in a dilute solution of a solvent such as water or benzene, the mass absorption coefficient of which is largely independent of wavelength\*; (4) relatively insensitive to the presence of impurities. In addition it is preferable that the reaction should be carried out in the presence of oxygen so that difficulties of degassing do not arise.

---

\* A reaction in, for example, carbon tetrachloride is useless since its photoelectric absorption per gramme is much higher than that of water, so that the amount of reaction per rad will vary with the voltage of the x-ray generator when this is less than 200 kV.

#### MEASUREMENT OF DOSE

Unfortunately no reaction has yet been found which fulfils all the requirements and is yet capable of measuring reasonably small doses. Probably the most satisfactory is the oxidation of ferrous sulphate which has been developed by MILLER<sup>13</sup>. This method is suitable for  $\alpha$ -,  $\gamma$ - and  $\beta$ -rays with energies above 50 keV. For radiations below this energy as well as for radiations of heavy particles the yield falls off.

Unfortunately the oxidation, though easily measured spectrophotometrically, cannot give accurate values for doses of less than 5000 r. Also, the reaction is extremely sensitive to the presence of impurities, which can both increase and decrease the amount of reaction. With due precaution, however, the method is reliable and far superior to any other at present available.

By calibrating the radiation source with an ionization chamber, a value of 6.5 atoms of iron oxidized per ion pair formed (or  $20.0 \pm 0.5$  atoms of iron per 100 eV of energy absorbed) has been obtained. In the last few years a number of attempts have been made, with powerful  $^{60}\text{Co}$  sources of  $\gamma$ -rays, to compare the amount of heat produced in water with the number of atoms of iron oxidized. In this way the chemical dosimeter would be calibrated directly in energy units, and would be independent of an ionization measurement. Although widely divergent values were obtained, the figure of HOCHANADEL<sup>14</sup> of 15.5 atoms oxidized per 100 eV of energy absorbed has been widely accepted. The difference between this value and that found using ionization chambers has been attributed to the fact that measurements of energy absorption made in gases could not be translated to energy absorption in liquids. It is questionable if the well substantiated view (see p. 24) that energy absorption per gramme is independent of the physical state of the absorbent, can be set aside on the basis of these experiments, since microcalorimetry is notoriously difficult to carry out. Moreover, in a more recent calorimetric determination<sup>15</sup> a value of 19 atoms of iron oxidized per 100 eV was found, which is very close to the 'ionization chamber value'. Our reason for referring to this controversy is that the ferrous sulphate dosimeter is widely used by radiation chemists, some of whom use the value of 20 and others 15.5. In comparing different results it is, therefore, necessary to ensure that the units used were based on the same definition.

#### IONIZATION DENSITY

*Effect of velocity and charge of ionizing particle*—Corpuscular radiations pass through matter in paths which are essentially straight for

### EFFECTS OF IONIZING RADIATIONS ON MATTER

a few microns, though electron tracks become detectably bent towards the end of their tracks as a result of scattering by atoms (see *Figure 8*). The energy dissipation of a corpuscular particle (*i.e.* the number of eV of energy lost per micron of track) is an inverse function of its total energy. Hence the number of ions formed per micron (this being the directly measurable process of energy dissipation) is also an inverse function of the initial energy. The ion density is not of course constant along the whole track, since with each ionization the ionizing particle loses energy, the specific ionization \* increases as the particle approaches the end of its range

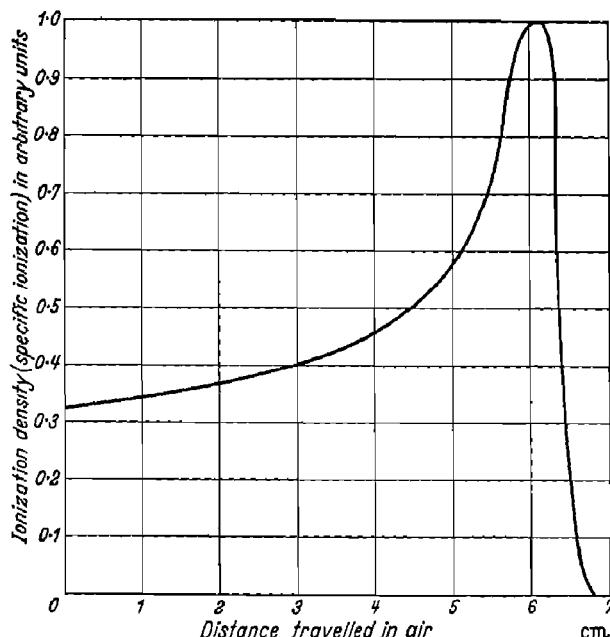


Figure 6. Variation in the ionization density produced by an  $\alpha$ -particle from RaC, with the distance travelled in air

and is at its maximum at the end of the track. An experimental demonstration of this is given in *Figure 6*; the final fall off in specific ionization at the end of the track is largely due to the capture of electrons by the  $\alpha$ -particles towards the end of their track. In this way the specific ionization is reduced because the charge of the particles is less.

\* Specific ionization is the number of ion pairs per unit length of track. Since the mechanism of energy loss is not clearly understood in condensed systems (see p. 23) the same effect is often expressed as the rate of loss of energy in eV (RLE) per unit length. It is usually assumed that  $RLE = 32.5$  (or 35)  $\times$  specific ionization.

### IONIZATION DENSITY

The specific ionization of charged particles is proportional to the square of their charge and inversely proportional to their velocity. Protons and electrons, therefore, moving at the same velocity will produce the same number of ions per unit length while an  $\alpha$ -particle with double the charge will produce four times as many. The velocity of particles having a given amount of energy is an inverse function of its mass, the exact relationship is complicated by relativistic effects. For example, a 200 MeV proton has the same velocity and, therefore, the same specific ionization as a 150 keV

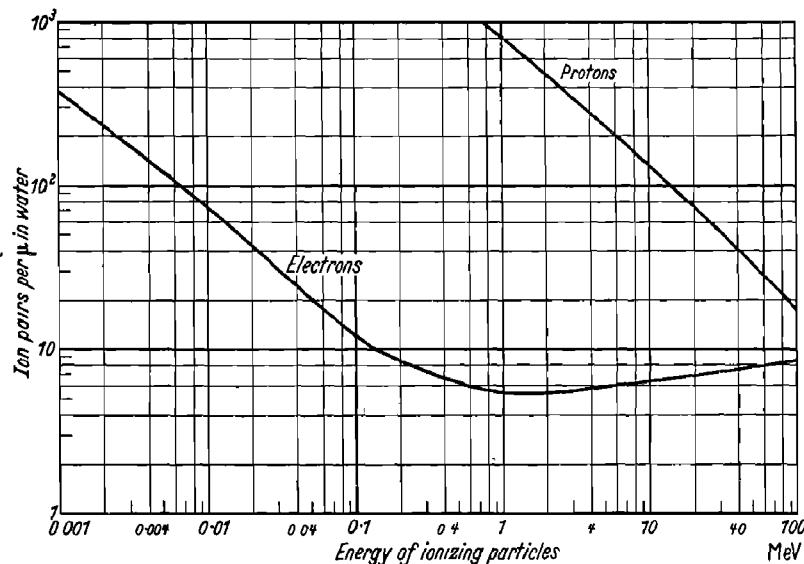


Figure 7. Ionization density (ion pairs per  $10^{-3}$  mm) in water of electrons and protons of different energy. (The ionization density was obtained by dividing the rate of loss of energy of the particle in eV per  $\mu$  by 32.5 for electrons and 35 for protons on the assumption (cf. p. 18) that these figures represent the energy in electron volts to form one ion pair. The data refers to the total energy loss which includes those produced by the primary particle as well as the secondary particles (e.g.  $\delta$ -rays). The actual ionization density along the track of the primary particle is about half the value shown. The difference being due to the secondary effects)

electron since the ratio of their masses is 1800 to 1. At comparable energies, therefore, the number of ionizations per  $\mu$  ( $10^{-4}$  cm) of track is very much greater (see Figure 7) for  $\alpha$ -particles and protons than for electrons.

All ionizations occur in the tracks of the ionizing particles. Thus tissue irradiated with a dose of 1 rep of electrons having the lowest specific ionization which is theoretically possible (see Figure 5) will contain a pattern of ions separated by  $0.2 \mu$  along the track and  $2 \mu$  between the tracks. On a molecular scale this inhomogeneity

## EFFECTS OF IONIZING RADIATIONS ON MATTER

is unimportant and in radiation chemistry this radiation will be homogeneous. With  $\alpha$ -rays, however, there will be many thousand ions per micron and for a comparable dose the tracks will be separated by centimetres. In the track ionizations occur within a few molecular diameters and the chemical effects produced by  $\alpha$ - and fast  $\beta$ -particles are, therefore, quite different.

*Distribution of ions in space*—Quantitative data concerning this complex problem are obtained in the Wilson cloud chamber. This instrument works on the principle that an ion causes precipitation to occur in a supersaturated system. When an ionizing particle passes through air supersaturated with water vapour minute droplets of water are formed round each ion and are photographically recorded as shown in *Figures 8–12*. From the experimental data obtained in this way and from the Bethe theory the rate of energy loss in gases and the associated quantitative specific ionization and range can be obtained.

The cloud chamber, however, is only useful for examining the track of an individual particle, and does not provide the complete pattern of the distribution of ions in space produced by a great number of particles; this involves three separate considerations: the energy spectrum of the ionizing particles, the direction of motion of the ionizing particles and the amount of ionization produced by particles of different energies. The problem is very complex, since the ejected electrons contribute so extensively to the observed ionizations, and even if the primary radiation is uniform, electrons of all energies will contribute to the overall effect. Still more complicated is the effect of x-rays and neutrons, which do not ionize themselves but produce a spectrum of ionizing particles.

Inspection of the Wilson cloud chamber photographs of electron tracks shows that at each ionization at least two water droplets are formed (see *Figure 11*). This is due to the fact that the ejected electron is captured by another atom thus producing a negative ion, and each ionization results in the formation of an *ion pair*. The electron, which has insufficient energy to produce ionizations, is known as a thermal electron and has a relatively short range in gases before it is captured by an atom or molecule to form a negative ion (*cf.* p. 34). From cloud chamber photographs in moist hydrogen gas the distance by which the positive and the negative ions of a pair are separated can be measured. The energy per ionization,  $W$  (*i.e.* 32·5 eV), refers to the formation of such an ion pair.

Often more than two droplets are formed close together, indicating the formation of several ion pairs at or near the same spot (see *Figure 11*). This cluster formation occurs because the electron, which

## IONIZATION DENSITY

is expelled as a result of the collision with the ionizing particle, can have sufficient energy to produce a few pairs itself. GRAY<sup>16</sup> estimates that for all ionizing particles approximately one-third of the ion pairs are produced directly (*i.e.* the ionizing particle passes through the atom which is ionized) and the remaining two-thirds are formed by electrons set in motion by the primary particle.

The range of these slow electrons, having energies of a few hundred electron volts, is very small (see *Figure 1*) and the ion pairs are, therefore, formed so close to the track of the primary particle as to be indistinguishable as a separate track, and on an average each cluster consists of three ion pairs. If, as happens occasionally, the electron ejected by the primary particle has an energy of several thousand electron volts, it forms a separate track and is sometimes referred to as a  $\delta$ -ray. The effect of these electrons, which are ejected in all directions from the track, is to introduce branching along a primary electron track. The branches are, of course, shorter than the track of the parent electron. With radiations of high specific ionization the total range of all the  $\delta$ -rays may be greater than that of the primary particle (*cf. Figure 9*). Inside this cylinder is a central core, the ionizing track of the heavy particle which has the high specific ionization. From this core emanate, in all directions, the  $\delta$ -rays which have a much lower ion density:  $\delta$ -rays introduce a general element of diffuseness into the distribution of the ions and this has to be taken into consideration in calculations concerning the target size.

*Ionization density in liquids*—All the data, both experimental and theoretical, concerning ionization densities apply to gases only. There is no method which closely corresponds to the cloud chamber for obtaining quantitative information of the formation of ions in condensed systems. However, the new technique<sup>17</sup> of detecting the tracks of ionizing particles in photographic emulsions, which was developed by Powell, is in many respects similar to the cloud chamber. In the so-called nuclear emulsion the ionizing particle renders the silver halide grains capable of being developed. From the amount of blackening along the track in the emulsion, particles with widely different specific ionizations can be distinguished and an estimate can be obtained of their range. The method is, however, severely limited and can provide absolute figures of the rate of energy loss per  $\mu$  of track only for particles of high RLE.

The values of specific ionization and range of ionizing particles in tissue (*i.e.* water) which are shown in *Figures 1, 7 and 14* are based on the assumption that a condensed system behaves in exactly the same way towards ionizing particles as a gas of the same atomic

## EFFECTS OF IONIZING RADIATIONS ON MATTER

composition. There are two assumptions, and they are nothing more, upon which all these calculations are implicitly based.

First, the energy dissipation of an ionizing particle is unaffected by the physical state of the absorbent (*i.e.* whether it is gas, liquid or solid) and is proportional to its density. GRAY<sup>18</sup> deduced from the available data that this is the case for  $\alpha$ -particles and there seems to be no reason to doubt that this principle can be extended to electrons and protons. The semi-quantitative data from nuclear emulsions on the range of different particles in gels are also in agreement when calculated on this basis. Second, the energy required to form an ion is the same in water and organic substances as in air. We know from direct experiment that the energy required to ionize an atom in air ( $W$ ) is 32.5 eV for electrons and 35 eV for the more massive particles such as protons or  $\alpha$ 's. No evidence is available to support the postulate that this value applies also in condensed systems. The use of the experimentally established value (see p. 18) for gases in water, tissue and other substrates can at the moment only be considered as an act of faith, founded on sound physical principles<sup>19</sup>. In view of the high yields, which have recently been reported for some radiation induced reactions in water, it is highly desirable to obtain some experimental evidence bearing on this point.

Another vital factor for the understanding of radiation processes in biological systems, about which we have no direct experimental evidence, is the separation of the positive and negative ions in water and tissue. LEA<sup>20</sup> and GRAY<sup>21</sup> have assumed that the behaviour of the thermal electron in the liquid phase is comparable to that in the gas phase. From this they calculate, taking the cloud chamber value of 20  $\mu$  for the separation in gases, that the positive and negative ions will be separated in water by 0.015  $\mu$  (150 Å). This calculation has, however, been challenged and both larger and smaller values have been proposed (see p. 85).

To summarize: it is reasonable to assume that the same amount of energy is lost by an ionizing particle when it traverses 1 g of water vapour or 1 g of liquid water, but the assumption, that the same number of ions is formed and that these occupy the same relative positions, is less well founded.

*Specific ionization produced by x- and  $\gamma$ -rays*—With x- and  $\gamma$ -rays the ionization occurs along the tracks of the ejected electrons, which start in a completely random manner, wherever an x-ray quantum has been absorbed (photoelectric effect) or scattered (Compton effect) (see *Figure 12*). The precise description of the irradiation with x-rays from a therapy set presents a problem of great magnitude,

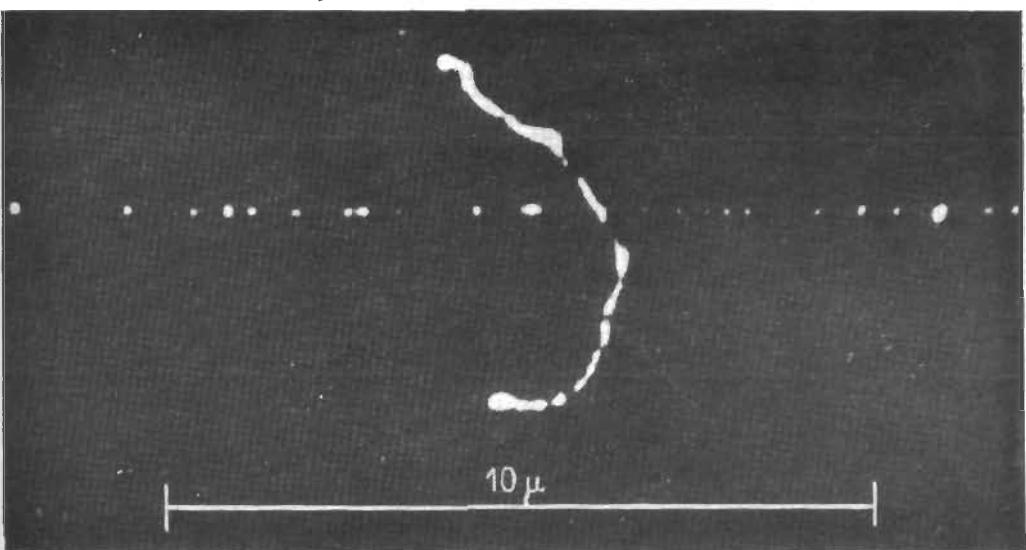
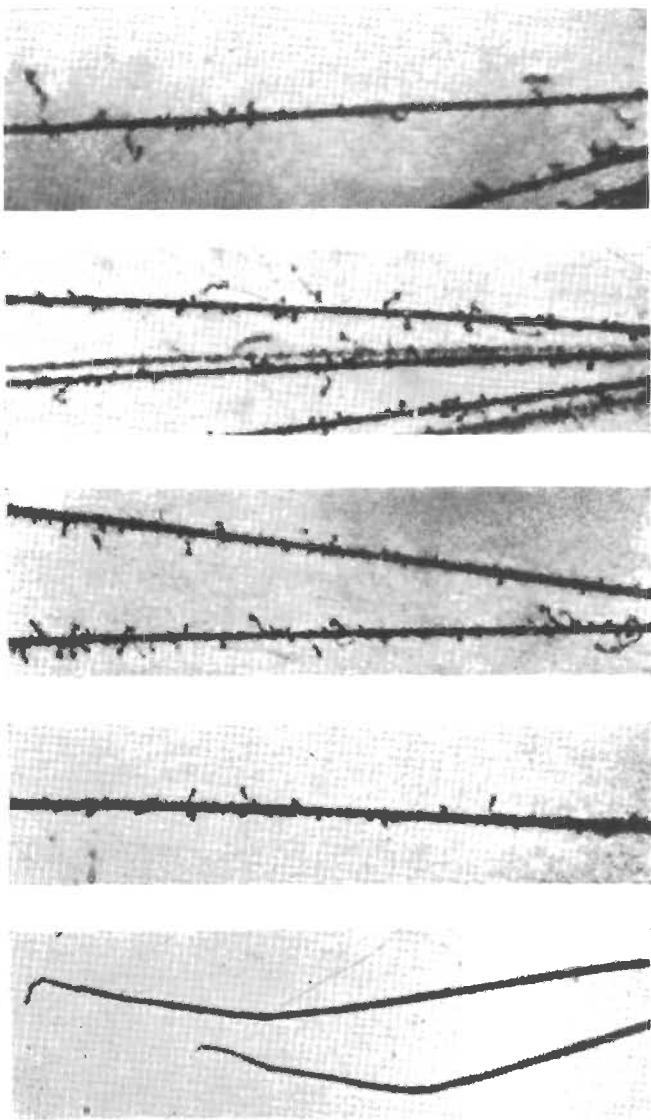


Figure 8. The track from left to right is that of a fast electron (circa 200 kV) in a cloud chamber. The bent track from top to bottom is produced by a slow 20 kV electron; at this low energy the track is bent because of scattering

Figure 9. Cloud chamber (b) tracks of  $\alpha$ -particles. Pictures of the early parts (i.e. high energy parts) in air (a) and (b) and helium (c) and (d). These clearly show the formation of  $\delta$ -rays, some of which have a considerable range. (e) Tracks at the end of the  $\alpha$ -particle range when  $\delta$ -rays are no longer formed. The tracks are bent at the very end, when their velocity is only of the order of  $10^8$  cm/sec, since the bulk of the energy of the  $\alpha$ -particles has been lost earlier. (Photographs reproduced by courtesy of Miss (d) T. Alper)



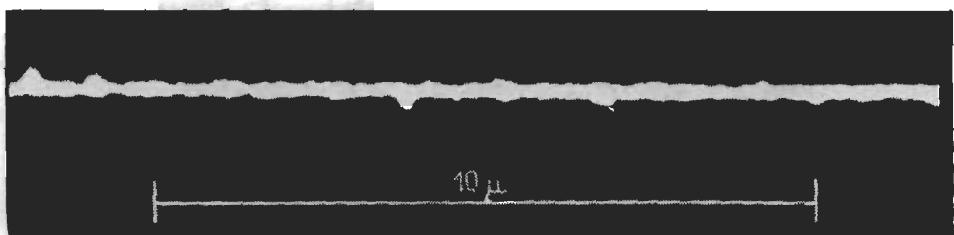


Figure 10. Cloud chamber track of a proton projected by a neutron

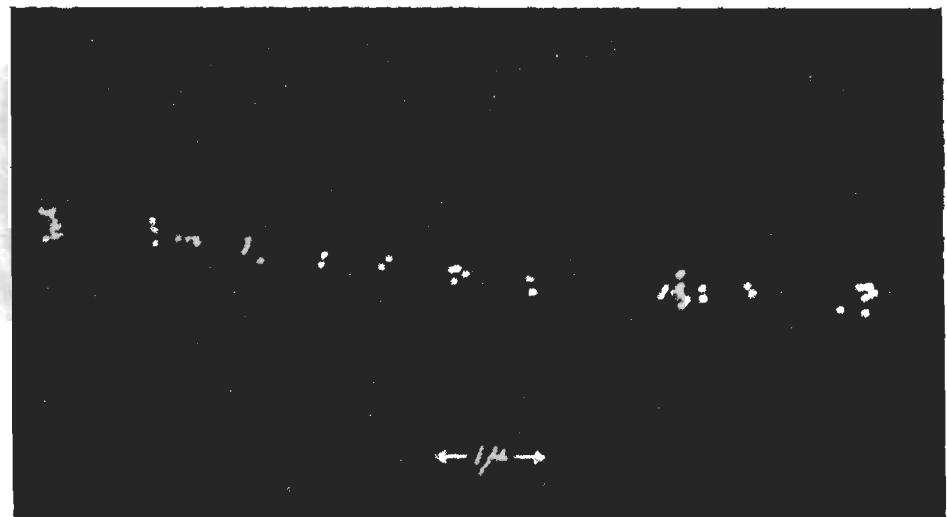


Figure 11. Cloud chamber track of a 1 MeV electron illustrating that ionization occurs in clusters. Successive clusters are separated by approx. 1  $\mu$ .

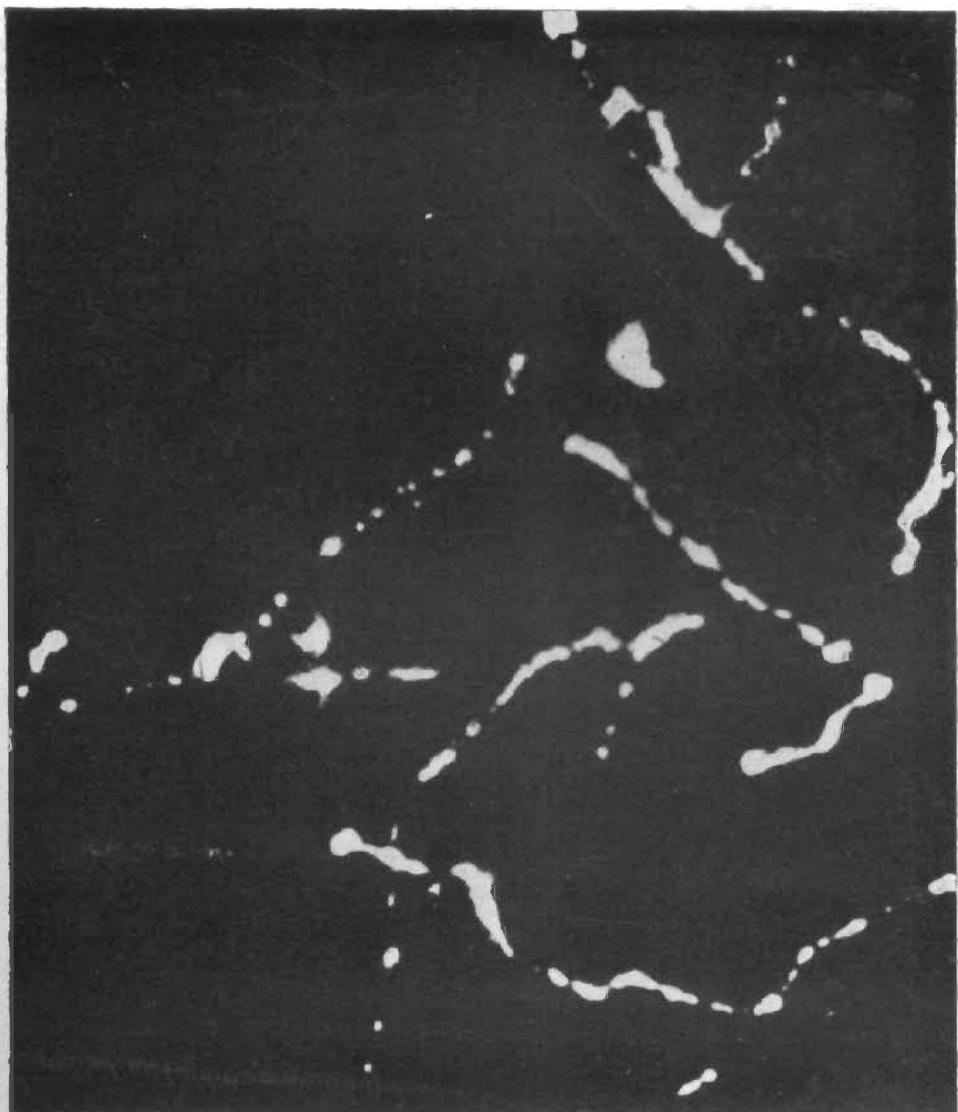


Figure 12. Cloud chamber photograph of the electrons produced by x-rays from a 200 kV therapy set

#### IONIZATION DENSITY

since an incident x-ray beam is usually inhomogeneous, and since, moreover, the beam may vary with depth in the irradiated material. In addition, at each wavelength a whole spectrum of ionizing electrons will be produced by the Compton effect. It is clear, therefore, that a volume of tissue irradiated by x-rays will be made up of tracks of electrons varying from a maximum energy (that of the peak voltage of the set used) to electrons of very low energy, including  $\delta$ -rays. Nevertheless, an average value for the specific ionization for x-rays produced at different voltages is of value.

The average value for the energy of the electron produced by

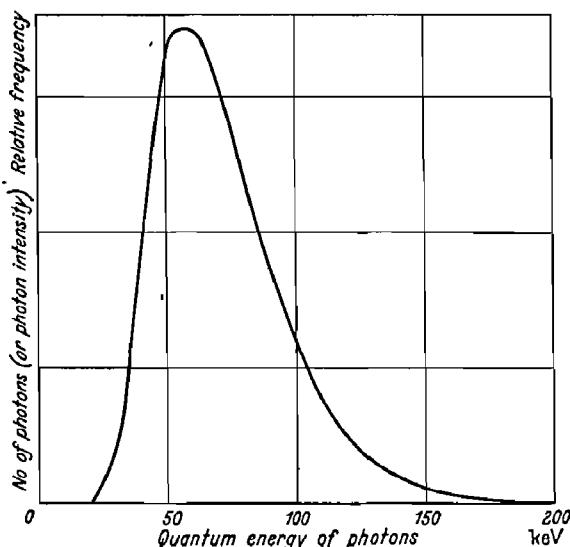


Figure 13. Spectrum of radiation from 200 kV x-ray therapy set. Curve reproduced from CORMACK and JOHNS<sup>22</sup>

homogeneous x-rays of different wavelengths have been computed by Lea. Using this data and coupling it with the known spectrum (see Figure 13) from an x-ray therapy set GRAY<sup>16</sup> obtained the energy distribution of the electron at a point, and by taking a direct average over a given volume the curve shown in Figure 14(a) for the change in average specific ionization with applied voltage was obtained.

There are many ways in which the different averaging processes can be carried out. CORMACK and JOHNS<sup>22</sup> as well as SPIERS<sup>23</sup> considered the spectrum (Figure 15) of all the electrons produced by the range of radiations given out by a therapy set working at a given

EFFECTS OF IONIZING RADIATIONS ON MATTER

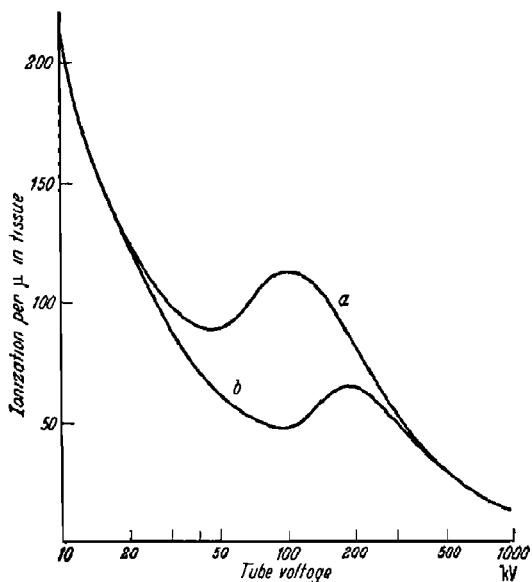


Figure 14. Relationship between the number of ionizations per  $\mu$  in tissue and the voltage of an x-ray therapy set; (a) Calculation based on the method of GRAY<sup>16</sup>; (b) CORMACK and JOHNS<sup>22</sup>

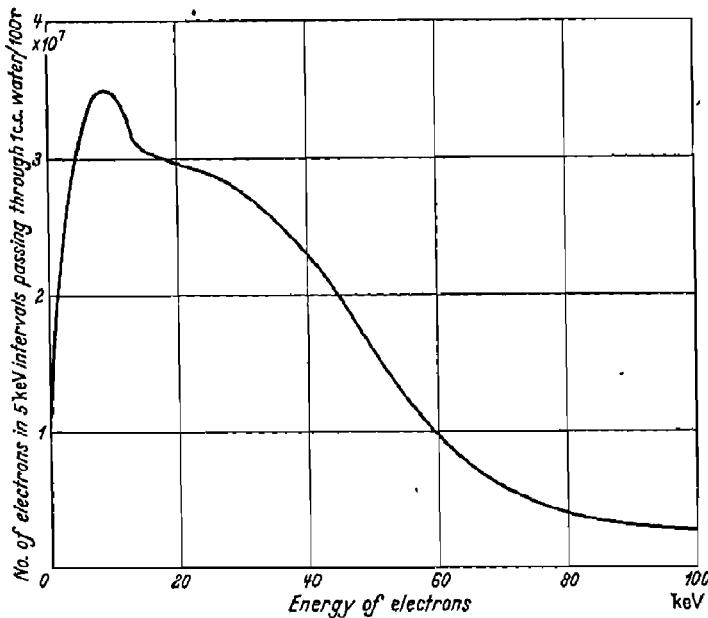
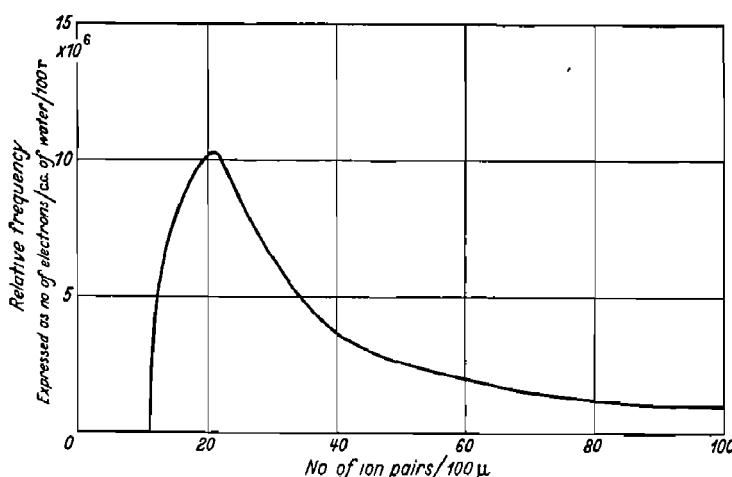


Figure 15. Distribution of energies of the electrons produced in water by the spectrum of radiation (cf. Figure 13) from a 200 kV x-ray therapy set. The results are expressed as the number of electrons, per 5 keV intervals, which pass through 1 cc of water irradiated with 100 r. (e.g.  $4 \times 10^6$  electrons having energies between 77.5 and 82.5 keV pass through each cc). Curve reproduced from CORMACK and JOHNS<sup>22</sup>

### IONIZATION DENSITY

voltage. From this energy spectrum the distribution of the number of tracks of different specific ionization was determined (see *Figure 16*) and an average obtained. By carrying out this procedure for x-rays produced at different voltages (*i.e.* by establishing *Figures 15* and *16* for different voltages), Cormack and Johns obtained the relationship shown in *Figure 14(b)* between average specific ionization and kV of the x-ray set. It will be seen that for radiations of less than 200 keV the average specific ionization obtained in this way is approximately half that found by Gray. The method of calculation of Cormack and Johns, though mathematically more precise, tends to mask the contribution of very low energy electrons (*i.e.* very short tracks of high specific ionization).



*Figure 16. Distribution of the ion densities of the electrons produced by a 200 kV x-ray therapy set. (This curve illustrates the difficulty of assessing the mean ion density and the limited significance of such a value for the ionizations produced with 200 kV x-rays.) Curve reproduced from CORMACK and JOHNS<sup>22</sup>*

The point to be borne in mind is that neither curve is wrong, but that in this highly complex system different treatments can lead to different results. The most meaningful value depends upon the purpose for which the calculation is required. Thus the Cormack and Johns treatment may be said to represent the track length average (*i.e.* it gives the mean number of ion pairs per  $\mu$  length of track) and should, therefore, be used for calculations of target size (see p. 56). The calculation of Gray gives the dose average (*i.e.* the number of ion pairs in a given volume of solution) which should be used for indirect processes when interaction between ion pairs is important (see p. 93).

### EFFECTS OF IONIZING RADIATIONS ON MATTER

The maximum in the curve is due to the fact that over this range the less energetic Compton recoil electrons are increasing in number while the more energetic photoelectrons are decreasing. Consequently the ion density only varies by plus or minus 10 per cent for

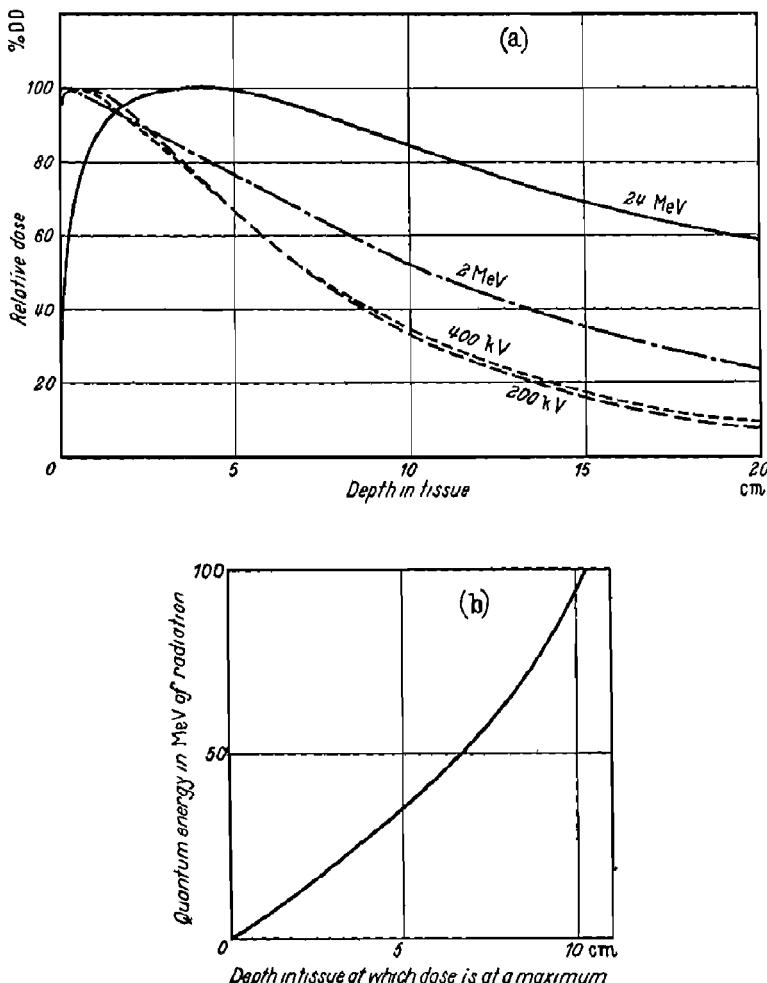
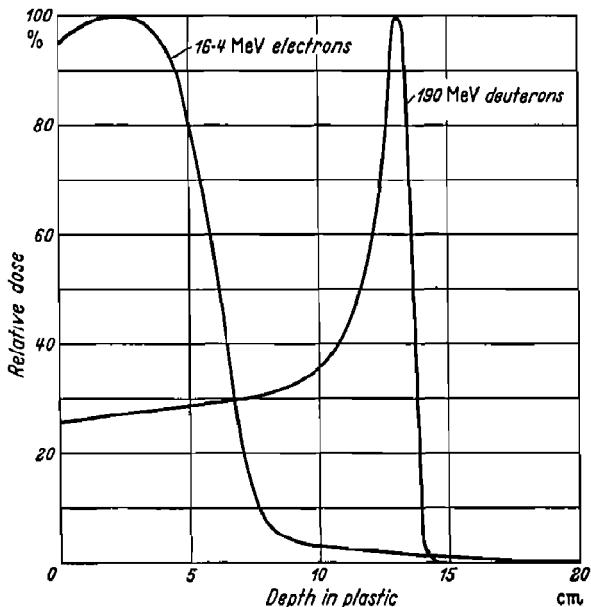


Figure 17. (a) Distribution of dose (i.e. amount of energy lost) inside tissue for radiations of different energies; (b) Relationship between the depth at which the dose is at a maximum and the energy of the radiation

x-rays produced by therapy tubes operating between 25 to 180 kV and experiments designed to determine the influences of ion density cannot be carried out by varying the wavelength within this range.

### IONIZATION DENSITY

An important feature of irradiation with very hard electromagnetic radiation, and one believed to be of therapeutic value, is that the maximum ion density is produced at a definite distance within the irradiated material (*e.g.* tissue). The reason for this behaviour is that the ejected electrons, which dissipate the energy, produce the highest specific ionization at the end of their tracks.\* Consequently electrons produced at the surface of the irradiated material give rise to the maximum number of ions at the end of their range. *Figure 17* shows the distribution of energy uptake inside



*Figure 18.* Comparison of the depth dose effect between deuterons and electrons. Curve taken from TOBIAS, ANGER and LAWRENCE<sup>24</sup>. These curves were determined experimentally using a plastic having an absorption coefficient similar to tissue

irradiated tissue for  $\alpha$ - and  $\gamma$ -rays of different energy. Once the maximum has been passed the intensity falls exponentially due to absorption (see p. 6).

For exactly the same reason the maximum energy loss of ionizing particles will occur at some distance within the irradiated material. Since particles have a fixed range—unlike electromagnetic radiations, which become progressively attenuated by absorption—the depth dose has a very sharp maximum. *Figure 18* shows

---

\* The increase of dose with depth is not observed with narrow electron beams because the effect of increasing ion density is more than offset by the divergence of the beam due to scattering.

## EFFECTS OF IONIZING RADIATIONS ON MATTER

experimentally determined distribution of energy curves for high energy electrons and deuterons.

*Multiple ionizations and Auger effect*—In the foregoing only the simple isolated ionization processes have been considered, in which energy is absorbed in ‘packets’ of 32·5 eV, and these are, of course, responsible for the predominant part of the energy absorption. There are, however, some infrequent events which probably do not contribute more than 2 or 3 per cent to the total energy taken up, in which much larger amounts of energy are released in one spot. One of these—the Auger effect—has been described (see p. 7); another is the multiple ionization in which more than one electron is stripped from an atom with which a primary particle collides. These rare events may be of biological significance, in particular for radiation with a low specific ionization, the average energy of which may be insufficient to disrupt, for example, a membrane, but this may nevertheless take place by one of the rarer events. This quantitative treatment of the ‘rare events’ of high energy transfer is difficult, and has been considered in detail by PLATZMAN<sup>25</sup> and GRAY<sup>26</sup>.

## EXCITATIONS PRODUCED BY IONIZING RADIATIONS

The energy transferred by ionizing radiations to matter is only in part used up in the formation of ion pairs, since for every one of these approximately 32·5 eV (or 35 eV for  $\alpha$ -rays and protons) is absorbed while the ionization potential for gases, determined in other ways, ranges from 24·5 V for helium to about 10 V for iodine and sulphur vapours; the value for air is 16 V and for water 13 V. For each ion pair formed in water vapour there is an excess of energy of the order of 20 eV, which is dissipated in excitations\*. According to the theory of Bethe an ionizing particle passes through a certain number of atoms without actually ejecting an electron, but displaces some of these from one shell to another of higher energy. It probably does this several times before it produces an ionization, and the total energy dissipated in all these processes including the latter is  $W$  (*i.e.* 32·5 eV).

The formation by  $\alpha$ -particles of excited atoms was first demonstrated in an impressive series of experiments by EYRING, HIRSCHFELD and TAYLOR<sup>27</sup>.

\* Excitation is used here to denote that an electron in the molecule has been raised to a higher energy level. This process is quite different from the excitation of a molecule by heating when the energy is used up initially to increase the strain in a bond by causing it to increase its vibrations and oscillations.

## EXCITATIONS PRODUCED BY IONIZING RADIATIONS

It should be stressed that this excess energy of 20 eV represents, in molecular terms, a great amount of energy (1 eV per molecule = 23.05 kcal per mole). Only 4 to 5 eV (*i.e.* approx. 100 kcal/mol) are required to break a carbon bond and if this excitation energy is transferred to a relatively few molecules many chemical changes per ion pair can be brought about. Unfortunately little is known concerning the mechanism of excitation by high energy electrons, and no information is available concerning the amount of energy transferred per excitation, or about the exact nature of the electron shifts produced. The possibility that the same atom which is ionized receives the excess energy as excitation is unlikely for simple molecules such as air or water, but may occur with more complex molecules.

Although both free radicals and excited molecules have, in general, only a transitory existence in condensed systems (usually between  $10^{-9}$  to  $10^{-6}$  sec) the reasons for their short life are not the same.<sup>1</sup> Free radicals are highly reactive entities, but they do not decompose or change spontaneously; in condensed systems free radicals have a short life (see p. 40) because they react so readily, and combination between radicals occurs, in many cases, on every collision. Excited molecules, on the other hand, are inherently unstable and must lose their energy by some process even if no collision occurs. In some cases excitation leads to immediate dissociation (*i.e.* within one atomic oscillation requiring  $10^{-13}$  sec). More often the excited molecule is stable for times greater than  $10^{-9}$  sec, after which it can undergo a delayed dissociation, lose its energy by giving off light (*i.e.* fluorescence) or undergo an energy transfer process.

Much is known concerning excitation of molecules by photons, and the same principles apply to excitation by electrons, although some of the strict selection rules which limit photochemical reactions need not be obeyed. The important Franck-Condon principle holds, however, and has a great influence in determining whether an excited molecule will dissociate immediately. The process can best be illustrated by means of a potential energy diagram of the diatomic molecule hydrogen iodide (see *Figure 19*). In its unexcited state the molecule HI has an internal energy corresponding to 'A', and the distance separating the two nuclei varies between  $a$  and  $a'$ .<sup>\*</sup> For dissociation to occur the potential energy of the molecule in its ground state has to be increased by an amount  $\Delta H$ ; in ordinary

\* Every molecule is always in a state of low activation at room temp. due to vibrational and rotational energy (zero point energy) and consequently the nuclei are not separated by the distance which corresponds to the minimum of the curve.

### EFFECTS OF IONIZING RADIATIONS ON MATTER

thermal dissociation this amount of energy is taken up as vibrational or rotational energy. The top curve represents a similar potential energy diagram for an electronically excited molecule. The dissociation energy of such a molecule  $\Delta H'$  is invariably smaller, and if it is of the order of the zero point energy instantaneous dissociation will occur.

Even if a molecule is relatively stable in the excited state, it may decompose instantaneously on becoming excited due to the operation of the Franck-Condon principle. This states that when an atom in a molecule is raised from one electronic level to another the posi-

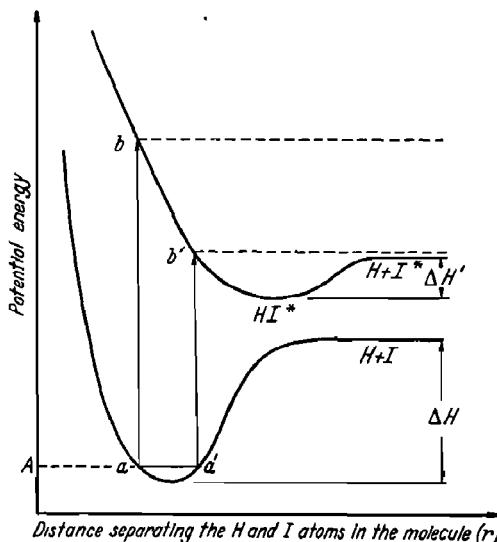


Figure 19. Potential energy diagram of (a) normal HI molecule and (b) electronically excited HI molecule

tion of the nuclei will not change (*i.e.* at least not appreciably). Consequently, when a molecule such as HI is exposed to ionizing radiations an electron of the iodine atom is raised to a higher energy level and the molecule is raised from its ground state (*i.e.* between points  $a$  and  $a'$ ) to an excited level with the same separation between the nuclei. It, therefore, falls on the corresponding potential energy curve between  $b$  and  $b'$ , where the energy is in excess of that required for dissociation, and immediate decomposition results. Thus, although an electronically excited HI molecule can be quite stable, it cannot be obtained in this form by excitation of HI. In agreement with these ideas LIND and LIVINGSTON found<sup>28</sup> an exceptionally high yield for the decomposition of HI by  $\alpha$ -articles.

## EXCITATIONS PRODUCED BY IONIZING RADIATIONS

H<sub>2</sub>I is not, of course, representative of all diatomic molecules and there are many molecules for which the excited state requires almost as much energy for dissociation as the ground state; such molecules almost invariably show strong fluorescence. An ionized molecule may also dissociate to give radicals, and from a chemical point of view it may be impossible to distinguish between radicals produced by ionization or excitation processes. In other cases only the ionized molecule is capable of giving free radicals the maximum yield of which cannot exceed the total number of ion pairs formed (*e.g.* polymerization of acetylene discussed on p. 38).

With more complex molecules excitation is unlikely to lead to immediate dissociation, since the excess energy can be distributed over many bonds, so that there is insufficient energy to break any one. An excited molecule of this type will have a relatively long life before the statistical event occurs in which the energy becomes localized and dissociation occurs. In the interval the excited molecule may lose its energy in a number of ways and return to the stable ground state. Since deactivating collisions are much more frequent in the liquid than in the gas phase, decomposition will be less frequent in a liquid, and this is amply borne out by experiment (see p. 39).

*Energy transfer*—In complex molecules the energy of excitation can be transferred both intra- and inter-molecularly\*. Energy can be transmitted from one molecule to another even when they are not in direct physical contact, by processes classed as radiationless transitions<sup>29</sup> which can only be described in quantum mechanical terms. In this way an electronically excited molecule can raise another molecule, separated by many molecular diameters, to an excited level. Energy transfer within the same molecule is known as internal conversion<sup>30</sup>; the electronically excited molecule returns to the ground state and the excess energy is converted into vibrational and oscillational energy. The molecule will then behave as if it were at a much higher temperature, and the irradiated substance will undergo changes similar to those occurring on pyrolysis.

In the present state of knowledge it is not possible to predict, in more than general terms, when either of these processes is likely to occur. It is unfortunate that excitation is much less amenable to quantitative treatment than ionization as this has led to an over-emphasis of the relative importance of the contribution of ionization in radiation processes: in biological systems the possible influence of excitation is rarely considered.

---

\* This type of energy transfer can only take place from a molecule in an electronically excited state. Vibrational or rotational energy of an energy rich molecule in the ground state cannot be transferred in this way.

## EFFECTS OF IONIZING RADIATIONS ON MATTER

### REACTIONS OF IONS

The ions, which are formed on irradiation, should strictly be called free radical ions since they always contain an uneven number of electrons; this distinguishes them from the stable ions produced by the dissociation of salts. The presence of an unpaired or odd electron makes free radicals, and the ions considered here, so highly reactive. A primary chemical bond can be represented as the sharing of two electrons between the constituent atoms and when two radicals collide such a bond is formed. This is the reason why simple free radicals have, in general, only a very short lifetime. Radicals obtained from complex organic molecules are often relatively long-lived because resonance stabilizes them and reduces the reactivity of the odd electron, which is distributed over the whole molecule. Radicals also have a great tendency either to lose or to gain another electron so as to have an even number when they finish as stable ions, e.g. the OH radical on capturing an electron becomes an OH<sup>-</sup> ion, which is one of the most stable entities known. Uncharged free radicals are written with a dot to designate an unpaired electron (e.g. OH').

*Negative ion formation*—The ‘thermal’ electron, ejected from an atom in a molecule which becomes a positive ion, must eventually either be captured by another molecule to give a negative ion or be recaptured by the positive ion. In the former reaction no great change in energy is involved, and the combined process is known as the formation of an ion pair. Charge neutralization, on the other hand, releases a large amount of energy.

The relative electron affinities of different molecules are known<sup>31</sup>, and if the electron does not recombine with a radical, this property determines with which atom it will combine. Molecular oxygen, and oxygen containing molecules such as water, have an extremely high electron affinity and in biological systems combination with any other atom is unlikely. The halogens have a high affinity; organic molecules, such as straight-chain paraffin, have a small affinity, while hydrogen and nitrogen can only combine with energetic electrons and will not compete for ‘thermal’ electrons.

The process of electron capture may be accompanied by dissociation, or the negative molecule may be stable,



Both the bond strength and the environment determine which

## REACTIONS OF IONS

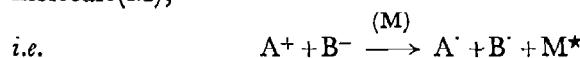
reaction occurs, e.g. the  $\text{H}_2\text{O}^-$  ion is stable in the gas phase, but dissociates in liquid water (see p. 83).

The subsequent behaviour of the negative ions is probably governed by the same general considerations as those of the directly produced positive ions, whose behaviour will now be described in general terms. Unfortunately experimental evidence for the different reactions is limited by technical difficulties, but the general concepts outlined below, formulated by Burton, Magee, Eyring and Platzman, are almost certainly correct and any mechanism proposed should conform to them.

*Charge neutralization*—The positive ion may capture an electron. This is almost bound to lead to immediate dissociation; the probable products<sup>32</sup> being two radicals, one of which is excited (excited products will be designated with a star),

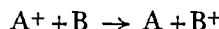


A positively and negatively charged (free radical) ion can only combine if a third molecule is present at the collision to take up part of the large amount of energy which is released<sup>33</sup>, otherwise immediate dissociation occurs. The presence of a 'third body' presents no difficulty in a condensed system, and a likely reaction is that the ions dissociate into two free radicals and activate the third molecule(M),



In general  $\text{M}^*$  will be less reactive than the free radicals produced.

*Charge transfer*—If the ionization potential of B is lower than that of A charge transfer will occur (for experimental evidence see pp. 38 and 42).



Theoretical considerations led BURTON and MAGEE<sup>34</sup> to the important conclusion that during a charge transfer process the two molecules involved stay together for as much as a second; such periods are long when considered in terms of chemical reactions. In this intermediate stage other molecules may become temporarily associated with the molecules undergoing charge transfer and thereby render possible more complex reactions. Modern quantum theory leads, therefore, to a return of one of the earliest theories for radiochemical reactions, which was proposed by LIND in 1919<sup>35</sup> (see p. 37).

*Dissociation of ions*—The free radical ion may be unstable and dissociate spontaneously in a number of different ways. Much information has been obtained for gaseous hydrocarbon from the

## EFFECTS OF IONIZING RADIATIONS ON MATTER

mass-spectrograph. WALLENSTEIN *et al.*<sup>36</sup> have examined this problem in great detail and predictions concerning the nature of the decomposition product can be made in simple cases, though not as yet for the more complicated systems likely to be encountered in radiobiology. In gases the following reactions occur and are illustrated on hand of a straight chain hydrocarbon:

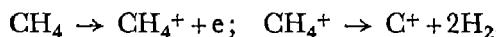


- (1)  $\text{C}_4\text{H}_{10}^+ \rightarrow \text{CH}_3^+ + \text{C}_3\text{H}_7^+$ . This is a dissociation into a free radical and a carbonium ion, which is not a free radical, but nevertheless unstable. It often rearranges to give off hydrogen and form an ion, which is stabilized by a double bond:



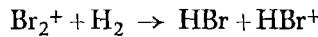
- (2)  $\text{C}_4\text{H}_{10}^+ \rightarrow \text{CH}_4 + \text{C}_3\text{H}_6^+$  i.e. formation of a stable molecule and an unsaturated free radical ion.

With small molecules a complete break-up of the following type sometimes occurs<sup>37</sup>:



In liquids positive ions probably undergo rather similar changes, but there is no way in which these can be determined experimentally. The nature of the early events can only be deduced in condensed systems from the nature of the final products.

*Reactions of ions with neutral molecules*—In general the chemical changes produced by ionizing radiations are ascribed to the free radicals formed in one of the reactions discussed above. Only rarely has any evidence been found for the direct reaction of an ion with a neutral molecule. In the gas phase the process



has been definitely established<sup>38</sup> and LIVINGSTON<sup>39</sup> believes there is some evidence for the reaction  $\text{CO}^+ + \text{CO} \rightarrow \text{C}^+ + \text{CO}_2$ .

### DEFINITION OF RADIATION-CHEMICAL YIELD

The amount of reactions per radiation dose is conventionally expressed in one of two ways. Firstly, as the number of molecules changed or produced (M) per number of ion pairs formed (N); secondly, as the number of molecules changed or produced for each 100 eV of energy absorbed and this is referred to as the 'G' value. There is probably little to choose between these two ratios in the case of gases where the energy required to form an ion pair (W) has been experimentally determined; where  $W = 32.5$  eV, 'G' =  $3. M/N$ . For reactions in liquids and solids the 'G' nomenclature is to be pre-

### DEFINITION OF RADIATION-CHEMICAL YIELD

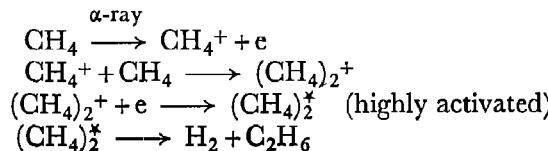
ferred, since only the total energy absorbed can be measured (see p. 23). However, when M/N values are quoted for reaction in liquids and solids it is always assumed that  $W = 32.5$  eV, so that all the data can be converted to 'G' values by multiplying by three.

### REACTIONS IN GASES

The chemical changes which are produced by exposing gases to ionizing radiations have been studied in great detail. The theoretical treatment of the results has been more successful than with other reactions, since the mass spectrograph can provide definite information concerning the type of radicals formed and their stabilities. Such data are not available for reactions in liquids or solids. The foundations for gaseous reactions were laid by the pioneers of atomic science; Mme Curie, Lord Rutherford, J. J. Thomson, W. Ramsay and F. Soddy. The whole of the earlier work is reviewed in a monograph by LIND<sup>40</sup>, who has himself made some of the outstanding contributions in this field; this book, though published 25 years ago, should be consulted by all interested in radiation chemistry. For the purposes of the present volume it is only necessary to refer to the salient features of gaseous reactions.

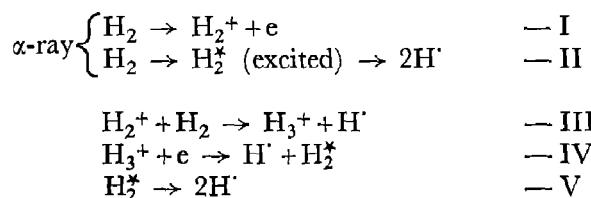
*High ionic yield*—Lind introduced the M/N nomenclature for expressing the yield of a radiochemical reaction. In all the gaseous reactions, such as oxidations, hydrogenations, decompositions, studied, M/N is only rarely less than 2. Often values ranging from 2 to 6, and occasionally even higher, have been reported. A value of two can be explained if it is assumed that both positive and negative ions are equally reactive, but this explanation cannot apply to gases with low electron affinities when a negative ion is not formed; or when M/N > 2. In a few cases high M/N are due to chain reactions, but detailed kinetic studies by LIND<sup>40</sup> have ruled this possibility out in the majority of cases.

LIND<sup>35</sup> interpreted the results by postulating the formation of clusters round the positive ion by polarization forces. The positive ion was then neutralized by the electron (or an oppositely charged ion) and the large amount of energy released shared by all the molecules in the cluster. The decomposition of a saturated hydrocarbon<sup>41</sup> was written as follows:



## EFFECTS OF IONIZING RADIATIONS ON MATTER

As more experimental evidence became available the cluster theory ran into difficulties and the suggestion by EYRING, HIRSCHFELDER and TAYLOR<sup>27</sup> has been widely accepted that the high yields in gaseous reactions are due to the fact that excited as well as ionized molecules can take a part in the chemical reaction. On irradiation of pure hydrogen with  $\alpha$ -particles, six hydrogen atoms were formed per ion pair and EYRING *et al.*<sup>27</sup> established the following reaction sequence:

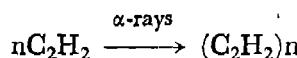


(i.e.  $M(\text{H}^\cdot)/N = 6$  (2 due to ionization + 4 due to excitation). Reaction III is an example of charge transfer accompanied by dissociation (see p. 35).

Additional evidence, that excited molecules produced by the passage of an ionizing particle through a molecule are responsible for many of the changes observed, was provided by SMITH and ESSEX<sup>42</sup>, who found that the  $\alpha$ -ray induced decomposition of nitrous acid and of ammonia was not greatly reduced when an electrically charged plate was introduced into the reaction vessel. Since ions will be attracted to the electrode, where they can be neutralized without dissociation, the small reduction in yield was attributed to the fact that a substantial part of the dissociation arose from excited molecules.

*Charge transfer*—LIND and BARDWELL<sup>41</sup> found that the presence of inert gases ‘catalysed’  $\alpha$ -ray-induced reactions. Both in polymerization reactions (*e.g.* acetylene, hydrocyanic acid and  $\text{C}_2\text{N}_2$ ) and decompositions (*e.g.* water, carbon dioxide and ammonia) the number of molecules changed was not altered by the presence of non-reactive gases, such as nitrogen, or one of the inert gases, even though the majority of the ionization occurred in the inert gas. Lind did not attribute this ‘catalytic’ effect to a transfer of electrons from the inert gas to the reactive gas, but postulated the formation of mixed clusters. Since the reactants in a charge transfer process are now known to form long-lived complexes (see p. 35) the two views can be reconciled.

For the polymerization of acetylene



## REACTIONS IN GASES

where  $M/N = 20$  the 'catalytic' effect of inert gases is very evident. LIND<sup>43</sup> believes that only the cluster theory can explain the fact that xenon, which has a lower ionization potential than acetylene, promotes the reaction as well as gases having higher ionization potentials. Only the latter can transfer charge to acetylene molecules. Steric considerations, however, exclude the formation of clusters of twenty molecules and according to EYRING<sup>44</sup> and STEACIE<sup>45</sup> the free radical ions (both  $C_2H_2^+$  and  $Xe^+$ ) initiate polymerization by a chain mechanism, which is well known to occur in other systems. The observation that benzene is produced as well as the polymer<sup>46</sup> also follows directly from the polymerization mechanism<sup>47</sup>.

*Summary*—Consideration of all the data indicates that radiation chemical processes in gases can best be interpreted in terms of the reaction of free radical ions and excited molecules. Some of these reactions, such as charge neutralization and charge transfer, lead, however, to complexes similar to those required by the cluster theory.

## REACTIONS IN LIQUIDS\*

Only indirect chemical methods are available for identifying the different ions formed when liquids are irradiated, and the reasonable assumption is usually made that the primary act is similar to that which occurs in gases. Since neither the energy to form an ion pair ( $W$ ) nor the ionization potential can be determined in liquids, it is postulated that the change from gaseous to liquid phase does not materially affect these values. Although there are bound to be small differences we assume that the energy to form an ion pair and its associated excitations is 32.5 eV and the ratio of energy expended as ionization and excitation is according to BURTON<sup>48</sup> approximately the same in liquids as in gases†.

A change in state, however, influences the secondary processes in a number of ways given below, and the final products in the

---

\* The important special case of water is dealt with in detail in Chapter 3.

† MAGAT *et al.*,<sup>49</sup> have devised two methods for determining the total number of free radicals (including ions) formed on irradiation of about 25 organic solvents with  $\gamma$ -rays. The 'G' values for radical formation (see p. 36) range from 0.85 for carbon disulphide, 10 for saturated hydrocarbons, 20 for acids and esters, to 60 for halogenated hydrocarbons. When 'G' exceeds 6, excitations must be responsible for some of the free radicals produced. Lower values are more difficult to interpret. The experimental techniques used in this pioneer investigation are liable to a number of errors, and the numerical values are, therefore, uncertain although the general pattern is clear.

## EFFECTS OF IONIZING RADIATIONS ON MATTER

condensed phase are often qualitatively and quantitatively different from those obtained in the gases.

(i) Deactivation of electronically excited molecules will be enhanced by the more frequent collisions which can occur in condensed systems. For example, energy transferred by an internal conversion process into vibrational or rotational energy can be dissipated by collisions.

(ii) Recombination of ions and radicals can, in general, only occur if a third molecule is present to take up the excess energy. Such processes are much more frequent in liquids than in gases and often result in the reversal of a radiation induced process so that no net chemical change is observed.

(iii) In polar liquids, particularly water, the ions and free radicals formed will be solvated, and consequently have entirely different properties from those in the gas phase. For example, the energy of combination of a hydrogen atom and a hydroxyl radical releases 350 kcal per mole, while in water, owing to hydration, only 14 kcal are given out. Similarly the  $\text{H}_2\text{O}^+$  ion, though relatively stable in the gas phase, may (or must, see Chapter 3) decompose in water as a result of hydration.

(iv) The surrounding molecules in a liquid or solid, besides tapping-off energy as considered under (i) and (ii) also greatly reduce the opportunity for fragments of a decomposing molecule to escape from one another's influence by the so-called Franck-Rabinowitch cage effect<sup>50</sup>. The solvent molecules 'bounce' the products back and thereby bring about recombination of radicals; the larger these are the smaller is the probability of escape<sup>51</sup>. In the 'cage' the radicals will recombine with loss of vibrational energy, and this process may be repeated until all the energy is degraded into heat\*. Hydrogen atoms and possibly small entities, such as the methyl radical, may escape from the cage with a high probability, and this explains the formation of crosslinks, which is observed when certain polymers are irradiated (see p. 121). With heavy ionizing particles the density of the ionizations will, to some extent, 'break-

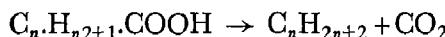
---

\* During an internal conversion process most of the energy is initially situated in one bond and this may explain why it is not always the weakest bond which breaks. The energy to break a C—C bond is 15 kcal per mole less than that to break a C—H bond, yet hydrogen is often produced in largest yield on irradiation of an organic material. The cage effect cannot be wholly responsible and the reaction probably results from the initial localization of energy in the C—H bond. In a liquid, where collisions are constantly occurring, the chance of sufficient energy appearing before deactivation in one bond of an energy rich molecule is small, and initial reactions (*i.e.* within  $10^{-12}$  sec) are more important in liquids than in gases.

## REACTIONS IN LIQUIDS

down' the cage, since active molecules will be produced in high local concentration; *e.g.* along an  $\alpha$ -track in a liquid the average distance between ionized molecules is of the order of ten molecular diameters. If one considers in addition excited molecules the distance between molecules undergoing change is not great and this may prevent the cage from being fully effective for densely ionizing particles.

All these factors combine to limit both the yield and the number of different products formed when liquids or solids, particularly of large molecules, are irradiated. BURTON<sup>52</sup> pointed out that the most probable reaction is a so-called 'slow' rearrangement leading to the formation of two stable products, which are not influenced by the cage. Few detailed studies have been carried out to test this idea, but the decomposition of long-chain fatty acids and of aliphatic alcohols by  $\alpha$ -particles can be interpreted along these lines<sup>53</sup>. In both cases the most striking effect is the simplicity of the products. The predominant reaction when fatty acids are irradiated is one of decarboxylation



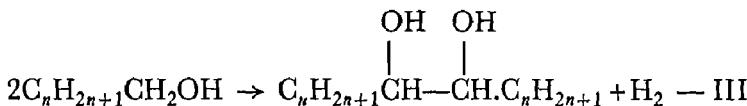
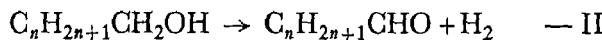
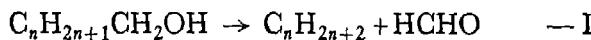
The only volatile product other than  $\text{CO}_2$  formed in appreciable quantity is hydrogen. The initial ionization and energy absorption will occur at random and in a molecule (*e.g.* palmitic acid) which on a weight basis consists predominantly of a hydrocarbon chain, one would expect this part of the molecule to be broken; in the gas phase random breakdown of hydrocarbons is observed.

In the liquid phase the non-specific nature of the energy dissipation by ionizing radiation is not apparent and reactions more reminiscent of photochemistry occur. This apparent contradiction is, of course, resolved when the cage effect is taken into consideration. The decarboxylation reaction is a rearrangement which is favoured by both steric and energetic factors. The presence of traces of hydrocarbons of varying chain length indicates that some reactive fragments escape from the cage, but that the probability is small.

The products obtained from alcohols are more complex, but McDONNELL's investigation<sup>54</sup> was sufficiently thorough to deduce the nature of the initial reaction from the final products. For straight-chain alcohols of the general formula  $\text{C}_n\text{H}_{2n+1}\text{CH}_2\text{OH}$  almost all reactions were confined to the  $\alpha$ -carbon (*i.e.* the carbon carrying the hydroxyl group). Although it was necessary to postulate many different reactions to account for all the products, in most

### EFFECTS OF IONIZING RADIATIONS ON MATTER

cases 90 per cent of the overall process occurred in the following three reactions



Reactions I and II are disproportionations giving stable products and, therefore, not affected by the cage. Reaction III is a 'cross-linking' reaction and results from the rupture of a C—H bond on the  $\alpha$ -carbon atom; the small hydrogen atom can escape leaving a free radical behind. In the densely ionizing track two such radicals then combine to give the product of reaction III. It is interesting that the yield of hydrogen is independent of chain length (in the range from  $n = 0$  to 9) with a 'G' value of 3 to 4.

*Energy transfer*—Several examples of energy transfer in solids and liquids exposed to ionizing radiations have recently been described. Those occurring in macromolecular systems are dealt with in Chapter 4. MANION and BURTON<sup>55</sup> found that energy initially absorbed by cyclohexane can be transferred to benzene in a mixture of the two liquids. Benzene is remarkably resistant to radiation<sup>56, 57</sup> probably because energy distribution is very rapid in an aromatic ring and localization of energy is reduced\*. Cyclohexane, on the other hand, is readily decomposed and the 'G' value for hydrogen production is 2·4; the corresponding figure for benzene is 0·06. In a mixture of the two hydrocarbons the yield of hydrogen is a small fraction of that expected from the cyclohexane alone. The interpretation advanced is that the ionized cyclohexane transfers its charge to (*i.e.* captures an electron from) benzene, which has a lower ionization potential; transfer of excitational energy also occurs, probably by collision. The transferred energy of either type is dissipated in the aromatic ring and produces much less hydrogen than it would have done from cyclohexane.

### REACTIONS IN SOLIDS

For organic solids the same considerations apply as for liquids, except that energy transfer processes, which do not require collisions

---

\* Deactivation by collisions must also play a part in the radiation resistance of liquid benzene, since in the gas phase the 'G' value for hydrogen evolution is five times that in the liquid. For cyclohexane the reverse is the case.

## REACTIONS IN SOLIDS

such as internal conversion and radiationless transition, play a proportionally larger part. For example, the decarboxylation of fatty acids by  $\alpha$ -rays occurs equally with liquid as with solid members of the series. The decomposition of organic macromolecules (see p. 126) can also be satisfactorily interpreted on the ideas developed for liquids.

On irradiating a crystal or semi-conductor the thermal electrons produced can either return to their position immediately giving off fluorescent light, or be confined in electron 'traps', which are often imperfections in the crystal lattice. These trapped electrons give rise to the so-called F-centres, which absorb light, often in the visible region when they give rise to colour. They represent stored energy, which can be released on heating, when the increased thermal motion permits the electron to return to its original ground state, and light is given off. This thermoluminescence represents only a minute fraction of the total energy absorbed by the crystal. MOREHEAD and DANIELS<sup>58</sup> found that during the bombardment with  $\alpha$ -particles the crystal lattice of certain minerals was destroyed, and no longer gave an x-ray diffraction pattern. The crystallinity, however, was restored by heating, and considerable amounts of energy were then released (*e.g.* 26 cal/g). It is not known whether energy can be stored in this way in organic crystals, but the possibility cannot be overlooked.

From the point of view of radiobiology, reactions occurring in gels are of particular importance since many biological structures, and in particular chromosomes and spindles (see Chap. 5) are gels of widely varying water content. It is unfortunate that most investigations of the effect of radiations on biologically important macromolecules, such as proteins or nucleic acids, have been carried out either on the dry material or in extremely dilute solutions, since there are indications that unexpected reactions occur in more concentrated solutions, and particularly in the gels. The rigidity of nucleoprotein gels is markedly reduced by surprisingly low doses of x-rays (see Chap. 4), and STEIN<sup>59, 60</sup> has found evidence that the oxidation reduction reaction, which methylene blue undergoes on irradiation, is different in gels from that in aqueous solution.

## REFERENCES

- <sup>1</sup> LEA, D. E., *Actions of Radiations on Living Cells*, Cambridge Univ. Pr., 1946
- <sup>2</sup> HEITLER, W., *The Quantum Theory of Radiation*, Oxford University Pr., 1954
- <sup>3</sup> KLEIN, O. and NISHINA, Y., *Z. Phys.*, 1929, **52**, 853
- <sup>4</sup> BETHE, H., *Hand-u. Jb. chem. Phys.*, 1933, **24**(1), 273

EFFECTS OF IONIZING RADIATIONS ON MATTER

- <sup>5</sup> GRAY, L. H., *J. chem. Phys.*, 1951, **48**, 174
- <sup>6</sup> Recommendation of International Committee, *Amer. J. Roentgenol.*, 1954, **71**, 139
- <sup>7</sup> MAYNEORD, W. V., *Brit. J. Radiol.*, 1940, **13**, 235
- <sup>8</sup> GRAY, L. H., *Proc. Camb. phil. Soc.*, 1944, **40**, 72
- <sup>9</sup> MAYNEORD, W. V., *Brit. J. Radiol.*, Suppl. No. 2, 1950
- <sup>10</sup> MAYNEORD, W. V. and SINCLAIR, W. K., *Advanc. biol. med. Phys.*, 1953, **3**, 1
- <sup>11</sup> GRAY, L. H., *Brit. J. Radiol.*, 1937, **10**, 600, 721
- <sup>12</sup> GRAY, L. H., *Proc. roy. Soc.*, 1936, **A156**, 578
- <sup>13</sup> MILLER, N. and WILKINSON, J., *Disc. Faraday Soc.*, 1952, **12**, 50
- <sup>14</sup> HOCHANADEL, C. J. and GHORMLEY, J. A., *Brookhaven Conf. Rep.*, Feb. 1952, p. 39
- <sup>15</sup> GOLDBLITH, S. A., PROCTOR, B. E., DAVISON, S., LANG, D. A., KAN, B., BATES, J. C., KAREL, M., *Radiology*, 1953, **60**, 732
- <sup>16</sup> GRAY, L. H., *Brit. J. Radiol.*, Suppl. No. 1, 1947, p. 7
- <sup>17</sup> YAGODA, H., *Radioactive Measurements with Nuclear Emulsions*, Wiley, New York, 1949
- <sup>18</sup> GRAY, L. H., *Proc. roy. Soc.*, 1928, **A122**, 648
- <sup>19</sup> Bibliography in MINDER, W. and LIECHTI, A., *Experientia*, 1946, **1**, 298
- <sup>20</sup> LEA, D. E., *Brit. J. Radiol.*, Suppl. No. 1, 1947, p. 59
- <sup>21</sup> DALE, W. M., GRAY, L. H. and MEREDITH, W. J., *Phil. Trans.*, 1949, **A242**, 33
- <sup>22</sup> CORMACK, A. and JOHNS, B., *Brit. J. Radiol.*, 1952, **25**, 369
- <sup>23</sup> SPIERS, F. W., *Disc. Faraday Soc.*, 1952, **12**, 13
- <sup>24</sup> TOBIAS, C. A., ANGER, H. O. and LAWRENCE, J. H., *Amer. J. Roentgenol.*, 1952, **67**, 1
- <sup>25</sup> PLATZMAN, R. L., *Symp. Radiobiol.*, p. 97, Wiley, New York, 1952
- <sup>26</sup> GRAY, L. H., Lectures in Paris ; to be published
- <sup>27</sup> EYRING, H., HIRSCHFELDER, J. O. and TAYLOR, H. S., *J. chem. Phys.*, 1936, **4**, 479
- <sup>28</sup> LIND, S. C. and LIVINGSTON, R., *J. Amer. chem. Soc.*, 1936, **58**, 612
- <sup>29</sup> FRANCK, J. and LIVINGSTON, R., *Rev. mod. Phys.*, 1949, **21**, 505  
FORSTER, T., *Ann. Phys., Lpz.*, 1948 (6), **2**, 55  
PERRIN, F., *Ann. Phys., Paris*, 1932, **17**, 283
- <sup>30</sup> TELLER, E., *J. phys. Chem.*, 1937, **41**, 109
- <sup>31</sup> MAGEE, J. L., *ibid.*, 1952, **56**, 555
- <sup>32</sup> MAGEE, J. L. and BURTON, M., *J. Amer. chem. Soc.*, 1950, **72**, 1965
- <sup>33</sup> BURTON, M., MAGEE, J. L. and SAMUELS, A. H., *J. chem. Phys.*, 1952, **20**, 760
- <sup>34</sup> BURTON, M. and MAGEE, J. L., *J. phys. Chem.*, 1952, **56**, 842
- <sup>35</sup> LIND, C. S., *J. Amer. chem. Soc.*, 1919, **41**, 551
- <sup>36</sup> WALLENSTEIN, M., WAHRHAFTIG, A. L., ROSENSTOCK, H. and EYRING, H., *Symp. Radiobiol.*, p. 70, Wiley, New York, 1952
- <sup>37</sup> SMITH, L. E., *Phys. Rev.*, 1937, **51**, 263
- <sup>38</sup> EYRING, H., HIRSCHFELDER, J. O. and TAYLOR, H. S., *J. chem. Phys.*, 1936, **4**, 570

#### REFERENCES

- <sup>39</sup> LIVINGSTON, R., *Biol. Antioxidants (Macey Conf.)*, 1952, **5**, 251  
<sup>40</sup> LIND, S. C., *The Chemical Effects of  $\alpha$ -Particles and Electrons*, Chem. Catalog. Co., New York, 1928  
<sup>41</sup> LIND, S. C. and BARDWELL, D. C., *J. Amer. chem. Soc.*, 1926, **48**, 2335  
<sup>42</sup> SMITH, C. and ESSEX, H., *J. chem. Phys.*, 1938, **6**, 188  
<sup>43</sup> LIND, S. C., *J. phys. Chem.*, 1952, **56**, 920  
<sup>44</sup> EYRING, H., *J. chem. Phys.*, 1939, **7**, 792  
<sup>45</sup> STEACIE, E. W. R., *J. phys. Colloid Chem.*, 1948, **52**, 441  
<sup>46</sup> MUND, W. and ROSENBLUM, C., *ibid.*, 1937, **41**, 469  
<sup>47</sup> ROSENBLUM, C., *ibid.*, 1948, **52**, 474  
<sup>48</sup> BURTON, M., *ibid.*, 1948, **52**, 566  
<sup>49</sup> PRÉVOST-BARNAS, A., CHAPIRO, A., COUSIN, C., LAUDER, Y. and MAGAT, M., *Disc. Faraday Soc.*, 1952, **12**, 98  
<sup>50</sup> FRANCK, J. and RABINOWITCH, E., *Trans. Faraday Soc.*, 1934, **30**, 120  
<sup>51</sup> NORRISH, R. G. W., *ibid.*, 1937, **33**, 1521  
<sup>52</sup> BURTON, M., *J. phys. Colloid Chem.*, 1948, **52**, 810  
<sup>53</sup> BREGER, I. A., BURTON, V. L., HONIG, R. E., SHEPPARD, C. W., *ibid.*, 1948, **52**, 551  
<sup>54</sup> McDONNELL, W. R., *Amer. Atom. Energy Com., U.C.R.L.* 1378, 1950  
<sup>55</sup> MANION, J. P. and BURTON, M., *J. phys. Chem.*, 1952, **56**, 560  
<sup>56</sup> GORDON, S. and BURTON, M., *Disc. Faraday Soc.*, 1952, **12**, 88  
<sup>57</sup> HENRI, V. and ERRERA, J., *J. Phys. Radium*, 1926, **7**, 225,  
<sup>58</sup> MOREHEAD, F. F. and DANIELS, F., *J. phys. Chem.*, 1952, **56**, 546  
<sup>59</sup> STEIN, G., *Disc. Faraday Soc.*, 1952, **12**, 227  
<sup>60</sup> DAY, M. J. and STEIN, G., *Nature, Lond.*, 1950, **166**, 146  
<sup>61</sup> BRUES, A. M., *Adv. Cancer Res.*, 1954, **2**, 177

## DIRECT AND INDIRECT ACTION

THERE are two distinct mechanisms by which a chemical change can be brought about by ionizing radiations: (*a*) *by direct action*, the molecule undergoing change itself becomes ionized or excited by the passage through it of an electron, and (*b*) *by indirect action*, in which the molecule studied does not absorb the energy but receives this indirectly by transfer from another molecule. The difference is particularly well defined when solutions are irradiated, and we shall confine ourselves to the biologically important case where water is the solvent. The radiation chemistry of water and aqueous solutions will be dealt with in Chapter 3, and for the present it is sufficient to note that the ionization of a water molecule leads to the formation of free radicals. These are chemical entities carrying a lone electron (see p. 34) which renders them extremely reactive and combination between two radicals occurs in most cases on every collision. Consequently, free radicals—particularly of the simpler type—have a very short life in solution. Their rate of disappearance depends both on the concentration of substrate with which they react and on the specific ionization of the radiation. The latter determines the local concentration of the radicals, which in turn controls the rate of radical recombination (see p. 95). In water it is unlikely that free radicals persist for more than  $10^{-5}$  sec and in many cases the time will be considerably shorter. For all practical purposes, therefore, the primary chemical changes are instantaneous, even when they involve the formation of a reactive intermediary.

There are several other mechanisms by which the energy transfer, characteristic of indirect action, can occur. For example, charge transfer processes, which occur in gases (see p. 38) result in reaction kinetics which are characteristic of indirect action. The lifetime of an ionized water molecule before it dissociates into a free radical is, however, only of the order of  $10^{-11}$  sec, and in this interval the probability of an exchange of ionization with a substrate molecule of lower ionization potential is vanishingly small. For this reason indirect action in aqueous systems is believed to be produced entirely by the free radicals formed from water, although the pos-

## DISTINGUISHING BETWEEN DIRECT AND INDIRECT ACTION

sibility that excited water molecules may take part in some reactions has to be considered (see p. 111).

### METHODS FOR DISTINGUISHING BETWEEN DIRECT AND INDIRECT ACTION

When a biologically active material such as an enzyme or a virus in a pure form is irradiated dry, the action of the radiations is by definition direct. If the material is dry but impure (*e.g.* containing extraneous protein) it is possible that energy absorbed by the impurity may be transferred to the active material, thereby augmenting the direct action. Evidence for energy transfer of this type has been found in synthetic macromolecular systems. With biological materials the same target size (see p. 56) has been found for pure as well as impure preparations<sup>1</sup> indicating that energy absorbed by the impurity does not enter into the reaction and that the process is direct.

When a biologically active material is irradiated *in vivo* or in aqueous solution the action can be either direct or indirect. The following tests can often be used to decide which mechanism is operative, although their application in biological systems is not always possible.

*Dilution effect*—In solutions a fixed number of free radicals is produced by a given dose. If the action is indirect, therefore, the number of molecules (or organisms) inactivated will be independent of concentration\* since a constant number of radicals are available for reaction. If the action is direct the number of enzyme molecules inactivated will depend on the number present in the irradiated volume and will, therefore, be proportional to the concentration. Thus, if the reaction is indirect the number of molecules which have been changed is independent of concentration and the *percentage* inactivation decreases with increasing concentration (*i.e.* the greatest relative change will be observed in the most dilute solution). Conversely, if the action is direct the same proportion of molecules will be changed whatever the concentration (*i.e.* the percentage inactivation is constant for a given dose). This dilution behaviour is illustrated diagrammatically in *Figure 1*, and was clearly demonstrated with dilute solution of pure enzymes (see *Figure 2*) by DALE, GRAY and MEREDITH<sup>2</sup>, whose work is fundamental to the whole conception of direct and indirect action.

\* If the concentration of the substrate is below a certain value (*e.g.*  $10^{-4}$  per cent in the case of carboxypeptidase (see *Figure 2*)) this no longer applies, since some of the radicals then react with one another and not with the dissolved material (see p. 97).

#### DIRECT AND INDIRECT ACTION

*Chemical protection*—The free radicals produced in water are highly reactive, and are not at all specific in the reactions they undergo. The presence of another substance may reduce the number of

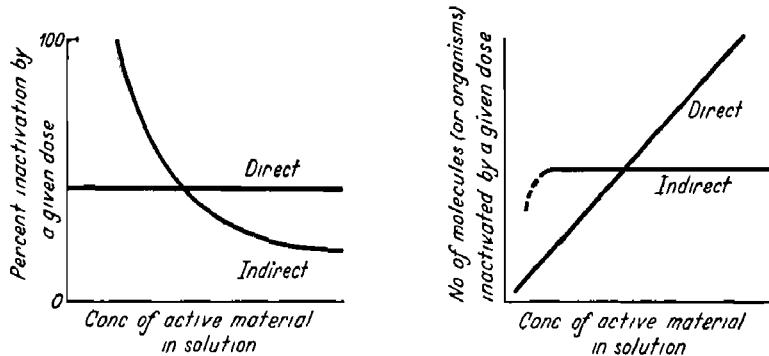


Figure 1. Dilution effect. The relationship between the inactivation of an enzyme or a virus and its concentration in solution depends on whether the action of the radiation is direct or indirect. The results can be expressed either as the percentage inactivation of the whole solution or as the number of macromolecules or organisms inactivated

enzyme molecules inactivated by competing with them for the limited number of free radicals available. Consequently, if the

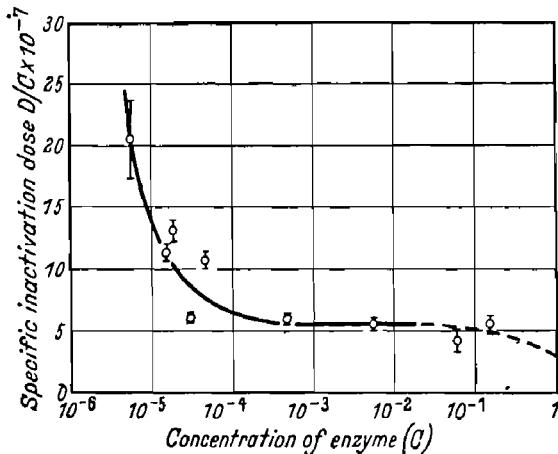


Figure 2. The relationship between the concentration ( $C$ ) of carboxypeptidase in aqueous solution (expressed as g of enzyme/g of enzyme + water) and the dose of x-rays ( $D$ ) required to reduce the activity of the solution to 37 per cent of its original value. For indirect action  $D/C$  should be constant. Except for the most dilute solutions this relationship is seen to be obeyed over an extremely wide range<sup>2</sup>

addition of another material reduced the amount of inactivation or other chemical change which is being measured (*i.e.* protects the system studied, see Chapter 14) the action of the ionizing radiations

## DISTINGUISHING BETWEEN DIRECT AND INDIRECT ACTION

is very probably *indirect*. This test, which was brilliantly developed by DALE<sup>3</sup>, is no longer quite infallible since it is possible to protect a macromolecule, which has been ionized or excited, by the addition of other substances to which it can transfer its energy before decomposition occurs (see p. 127). An added substance can also protect (*i.e.* reduce the observed radiation induced effect) by reacting with the molecule which has been damaged either directly or by free radicals and thereby prevent its inactivation<sup>78</sup> (for an example see p. 131). In biological systems, where the action is known to be direct, protection by extraneous materials has not been observed.

*Freezing test*—A most effective method of deciding if the action of radiation on a solute is direct or occurs via free radicals is to compare the effectiveness of a given dose at ordinary temperatures with that found at low temperature when the solution is frozen. In the latter case the diffusion of free radicals is hindered, and reaction by indirect process should be very greatly reduced\* while direct action will, in general, be much less dependent on temperature. Few biological systems survive freezing, but in the cases where the test can be used it proved most valuable.

## RELATIONSHIP BETWEEN RADIATION DOSE AND OBSERVED EFFECT

For indirect processes the relationship between dose and observed chemical change (for instance inactivation) depends upon the reaction product (see *Figure 3*).

(i) If this does not react with free radicals (as, for example, in simple reactions such as the oxidation of ferrous to ferric ions or the decomposition of formic acid), then the number of molecules changed will be directly proportional to the dose.

(ii) In reactions, such as the inactivation of enzymes or the degradation of macromolecules where the product (*e.g.* the inactivated enzyme) is still capable of reacting with free radicals, it will act as a protective agent. As the reaction proceeds the extent of protection increases and the dose curve will be exponential. If the product has the same reactivity with free radicals as the starting material (and this is often the case with enzymes) then a dose of radiation, which produces sufficient radicals to inactivate every enzyme molecule, will only bring about 63 per cent inactivation since 37 per cent of the radicals will react with molecules which

---

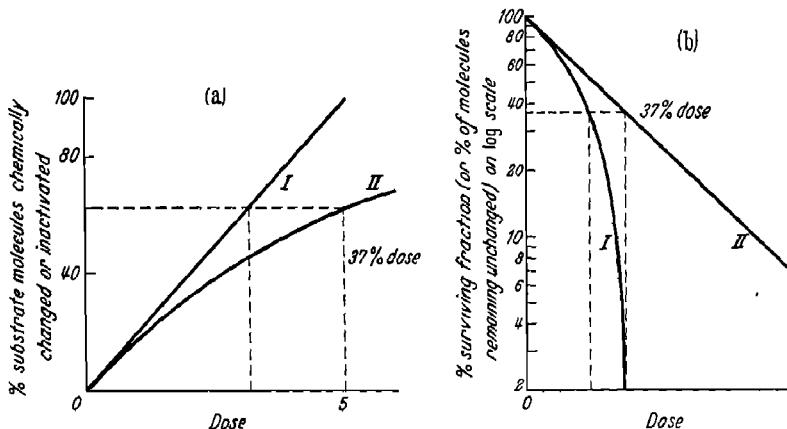
\* In general, the material is irradiated in the frozen state but examined subsequently when the ice is molten. There is no evidence that any free radicals formed in the irradiated ice become available for reaction on melting.

### DIRECT AND INDIRECT ACTION

have already reacted once. For this reason the terminology used by LEA<sup>4</sup> to express the sensitivity of an organism or enzyme to radiation as the 37 per cent dose (*i.e.* the dose when 37 per cent survive) is most useful:

The 37 per cent dose/number of organisms irradiated =  
dose required to inactivate one organism

It must be stressed that the 37 per cent dose has no meaning in complex systems such as whole-body irradiation of mammals where the radiation effect merely initiates a long sequence of events. In experiments where the required exponential relationship applies it is not necessary to use doses which inactivate as many as 63 per cent, since this value can be derived from the logarithmic plot (*Figure 3(b)*).



*Figure 3. Relationship between dose of radiation and change observed (e.g. percentage of molecules chemically changed or inactivated or number of organisms killed) for indirect action*

*I Where product does not react with the free radical responsible for the change (e.g. oxidation of  $\text{Fe}^{++} \rightarrow \text{Fe}^{+++}$  cf. p. 105).*

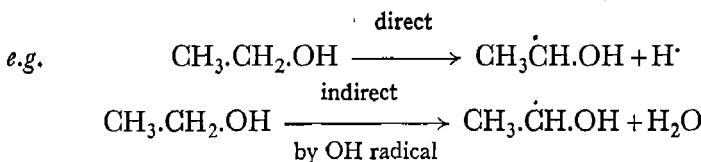
*II Where product reacts as readily as the original material with the free radicals (*i.e.* the radiochemical product acts as a protector). This is the case for the inactivation of carboxypeptidase in solution: (a) plotted linearly; (b) plotted logarithmically.*

For direct action of the simplest type (see p. 55) the relationships between dose and inactivation represented diagrammatically in *Figure 3* apply, since ionizations will be wasted by reaction with molecules or organisms which have already been inactivated. There is no difference between the dose versus effect curves for direct and for the most common form of indirect action (*i.e.* where the inactivated molecule competes for the radicals and thereby protects the remaining active units).

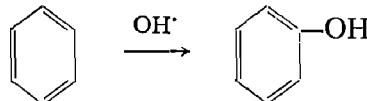
## RELATIVE EFFECTIVENESS OF DIRECT AND INDIRECT ACTION

### RELATIVE EFFECTIVENESS OF DIRECT AND INDIRECT ACTION

In many cases an enzyme or virus can be inactivated both by direct and indirect action, although the efficiency (or energy required) of the two processes is not usually the same. The initial reactions will be, of course, very different in the two cases: direct action usually brings about the decomposition of the molecule, while indirect action involves a free radical, which can either add on to the molecule or abstract from it an atom or group. If the free radical abstracts an atom then the final products obtained by direct and indirect action may be similar. This, for example, occurs with alcohols where the  $\alpha$ -carbon atom is activated by direct action and by indirect action suffers abstraction of hydrogen by an OH radical; the 'G' value in the two cases being approximately the same.



For benzene the position is reversed, the major product from indirect action in aqueous solution is the addition of a hydroxyl radical to give phenol



while irradiation of the pure liquid (direct action) gives a large number of different substances, including hydrogen, acetylene and a polymer. The energy required to form one molecule of phenol is approximately 45 eV (*i.e.* 'G' value = 2.2) while by direct action approximately 50 to 100 times the amount of energy is required to disrupt one molecule of benzene<sup>6</sup>. In general, the radiation resistance of molecules to direct action is not as great as for benzene, and in some cases the energy required to change a molecule chemically (though not necessarily to the same product) is of the same order of magnitude for direct and indirect action. This is illustrated by the degradation of certain synthetic polymers (*e.g.* polymethacrylates) where the energy required to break a main chain is the same for irradiation in aqueous solution as for irradiation of the dry material (see p. 125).

On the other hand, in many of the cases where a comparison is possible, the energy required to inactivate an enzyme or virus molecule when dry or highly concentrated (*i.e.* by direct action) is

### DIRECT AND INDIRECT ACTION

very much less than that required to inactivate it in dilute solution<sup>5</sup>. In general, one ionization per molecule\* is sufficient to inactivate a virus or a phage (see p. 61) by direct action, while more than a thousand ionizations may be needed for inactivation in dilute solution.

The dose required to inactivate a given proportion of a pure enzyme or virus will decrease with decreasing concentration owing to the contribution of the indirect effect. If  $D_0$  is the 37 per cent dose for direct action, which is, of course, independent of concentration, then the 37 per cent dose ( $D_c$ ) at concentration  $C$  (g solute/ml) is given by

$$D_c = \frac{D_0}{1 + \alpha/c}$$

where  $\alpha$  is the ratio of the energy required to inactivate by indirect action (*i.e.* number of solute molecules inactivated per ionization† in the solvent) over that required for direct action (*i.e.* number of molecules inactivated per ionization directly produced in the solute).  $D_c$  tends to  $D_0$  at high concentration and when  $\alpha$  is small (*i.e.* when direct action is more efficient than the indirect)  $D_c = D_0$  even in relatively dilute solutions. *Figure 4* shows a general curve relating the 37 per cent dose ( $D_c$ ) with concentration; at very low concentrations the inactivation dose does not continue to diminish, but reaches a limiting value because of radical recombination. That is, the concentration of the substrate is so low that active radicals are lost by reacting with one another instead of with the solute. Under those conditions which have been treated quantitatively<sup>5, 7</sup> the yield for indirect action—and, therefore, also the coefficient  $\alpha$ —falls with decrease in concentration. The half-way point, where the 37 per cent dose =  $\frac{1}{2}D_0$ , is reached when the concentration in g of substrate per ml is numerically equal to  $\alpha$ .

When impure preparations are irradiated the extraneous matter, which acts as a protective agent by competing for the available free radicals, reduces the yield of the indirect effect and consequently the value for  $\alpha$  decreases. For tobacco mosaic virus—as for most

\* The number of ionizations cannot be measured (see p. 23) but is calculated from the amount of energy absorbed by postulating that the value for  $W$  (the energy required to form an ion pair) is the same in proteins and nucleic acids as in air, where it has been determined (*i.e.* 32.5 eV for electrons and 35 eV for heavier particles).

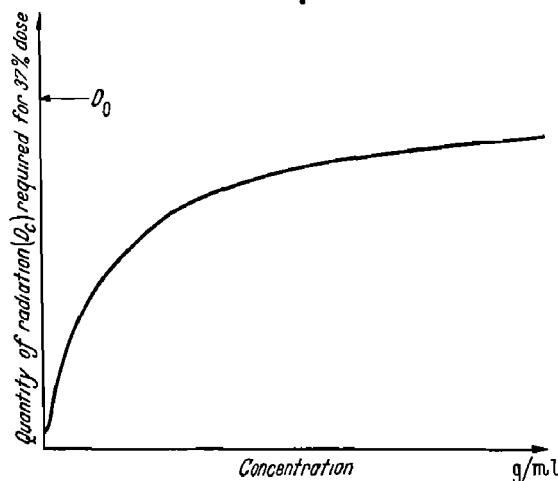
† Lea, who developed this subject, always used the M/N terminology (see p. 36) and assumed that the energy required to form an ion was the same for all materials. In fact all measurements are based on energy absorption and ionic yields can readily be converted to eV/per molecule. In any case the ratio  $\alpha$  is independent of the way in which the yield is expressed.

#### RELATIVE EFFECTIVENESS OF DIRECT AND INDIRECT ACTION

viruses—indirect action is very inefficient and  $\alpha$  is of the order of  $10^{-4}$  and the 37 per cent dose, therefore, only becomes dependent on concentration when this falls below  $2 \times 10^{-3}$  g/ml (see *Table I*)<sup>8</sup>. The effect of adding small quantities of gelatine (a protector) is to lower the indirect action still further, which then fails to contribute to the inactivation even at the highest dilution which could be used (*i.e.* the 37 per cent dose was independent over the whole concentration range).

For enzymes the yield for indirect action is much greater than for viruses and ranges approximately from  $10^{-2}$  to 1 (see Chapter 4). The value of  $\alpha$  is correspondingly higher and in solutions normally encountered (*e.g.* 1 per cent or less) the indirect effect makes a substantial if not the major contribution to the inactivation produced

*Figure 4. Influence of concentration on the inactivation dose (expressed as 37 per cent dose) for a material which can be inactivated by direct and by indirect action.  $\alpha$  is the ratio of the energy required to inactivate by indirect action divided by the energy to inactivate by direct action. The actual curve does not pass through the origin since at very low concentration the efficiency of indirect action falls due to radical recombination*



by ionizing radiations. Again the presence of other substances will act as protective agents but their effectiveness cannot be predicted. The rate of reaction of different materials with free radicals varies at least by a factor of  $10^4$  (see p. 96) and the value of  $\alpha$  will therefore depend entirely on the nature of the protective agents present.

Since the extent of protection which occurs *in vivo* is not known, data derived from *in vitro* experiments cannot be applied directly. The data indicates, however, that direct action is more likely to be important for the inactivation of virus than for enzymes. It may well be that the difference in the values between these can be attributed to the fact that the former are made up of giant molecules of nucleoprotein, whereas enzymes are proteins of intermediate molecular weight. The vitamins, ascorbic and nicotinamide, are

DIRECT AND INDIRECT ACTION

*Table I. Direct and indirect action in aqueous solution; x-rays on tobacco mosaic virus<sup>5</sup> and electrons on vitamins<sup>21</sup>*

Virus	Concentration in g/ml of		37 per cent dose $\times 10^{-5}$ r
	Protective agent		
Solid	—		2.5
0.14	—		2.9
0.022	—		2.9
0.00022	—		1.5
0.000022	—		0.5
0.0000044	—		0.6
0.000022	0.05 glucose		0.5
0.000022	0.001 glucose		2.4
0.000022	0.01 gelatine		2.4

Vitamin	Dose (rep $\times 10^{-6}$ )	Temp. °C	Protective agent	Decrease in activity
$1.3 \times 10^{-3} \%$ ascorbic acid	1	-35	—	8
" "	4	+11	—	27
" "	1	+18	—	37
" "	4	+18	—	84
" "	4	-35	orange juice	11
" "	4	+18	" "	19
$1 \times 10^{-4} \%$ nicotinamide	4	-35	—	12
"	4	+18	—	79

inactivated, when dissolved in water, to a much greater extent at  $4^\circ\text{C}$  than at  $-35^\circ\text{C}$  (see *Table I*). The small effect at the low temperature can be attributed to direct action. When these substances are dissolved in orange juice instead of in water, inactivation at  $18^\circ\text{C}$  is very much less, but the influence of temperature disappears almost entirely. Presumably the orange juice acts as a protective agent and reduces the indirect but not the direct effect.

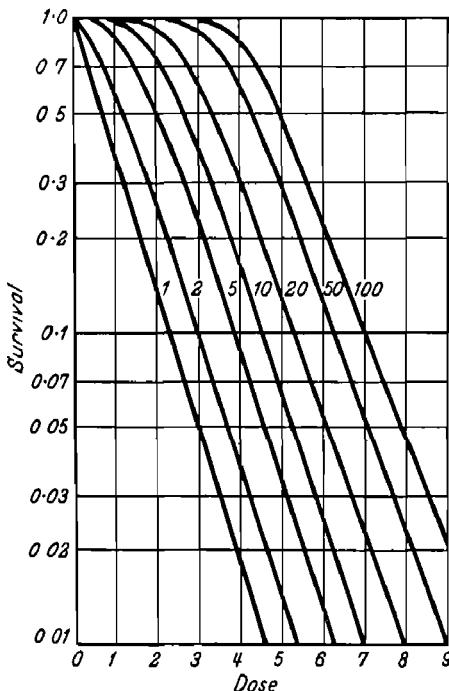
THE TARGET THEORY

In 1924 CROWTHER<sup>9</sup> found that the data relating inhibition of cell division with dose could be quantitatively interpreted if mitosis arrest was the result of a single ionization in a volume which corresponded to the size of the centromere. Crowther developed this theory to include effects where more than one ionization per organism was necessary for inactivation and showed how both the volume of the target and the number of ionizations could be calculated from the dose inactivation curve. This multi-hit theory implies that

## THE TARGET THEORY

more than one target has to be hit to produce the observed effect. *Figure 5* shows mortality dose curves which could be interpreted as requiring different numbers of hits. Since Crowther proposed this method, many workers have analysed biological results in terms of target size and numbers of hits. The discovery by DALE<sup>3</sup>, that indirect action via energy absorbed in the water may play an important part in the irradiation of biological materials, makes it necessary to establish that the process occurs by direct action before the target theory can be applied.

Largely as the result of the work by LEA<sup>4</sup> and his colleagues it



*Figure 5. Theoretical survival curves plotted on a log scale for materials requiring different numbers of hits for inactivation*

became clear that when a biologically active macromolecule is irradiated *in vitro* under controlled conditions where it can be proved that direct action predominates (*i.e.* dry or in solution sufficiently concentrated or protected to make  $D_e \sim D_0$ ) the results can be interpreted by the target theory and inactivation usually occurs as the result of a single ionization (see footnote, p. 52). From radiation experiments it is possible to determine the size of the target, and this is usually defined as a sensitive volume, within which any chemical change results in loss of biological activity.

Inherent in the target theory are the assumptions (i) that when an atom becomes ionized the molecule of which it is a part is chemically transformed, and (ii) that most, if not all, of the chemical

## DIRECT AND INDIRECT ACTION

changes produced by ionizing radiations within solids and liquids are due to ionization, and that excitation can be neglected. In the preceding chapter it has been shown that neither of these assumptions is necessarily valid; some substances, particularly those containing aromatic rings, are very radiation resistant and considerably less than one molecule is changed per 32.5 eV absorbed (*i.e.* per ionization) while for others the yield is much higher indicating that excitation plays a part. In spite of these objections the theory that biologically important molecules can be inactivated by a single ionization anywhere within their volume—the single-hit target theory—has been singularly successful. To be quite reliable it is preferable to determine target size from data obtained with radiations having a high specific ionization. In this way many ionizations providing ample energy for inactivation will occur whenever an ionizing particle traverses the target.

*Calculation of target size*—When the single-hit target theory applies, the inactivation must be exponentially related to the dose (see *Figure 3(b)*) and independent of the dose rate. A most critical test is that the quantity of radiation required to produce a given amount of inactivation must depend upon the specific ionization of the radiation used, those having the lowest ion density being the most effective, because the chance of producing more than one ionization (*i.e.* wasteful ionizations) within the target is smallest. A 5 MeV  $\alpha$ -particle will produce about 50 ionizations when passing through a target of 100 Å diameter, and consequently 98 per cent of the ionizations are wasted when inactivation is due to a single ionization. When more than one hit per organism is required the survival curve will be sigmoid and more densely ionizing radiation will be more effective (though there will be a maximum) than  $\gamma$ - or x-rays, since the chance of producing more than one ionization within a given volume will be greater. Although these relationships are often found *in vivo\** experiments, these cannot be analysed by the target theory, unless the essential premise of direct action has been established. In this section *in vitro\** experiments only are considered and when these are direct there is no evidence for any multi-hit effects.

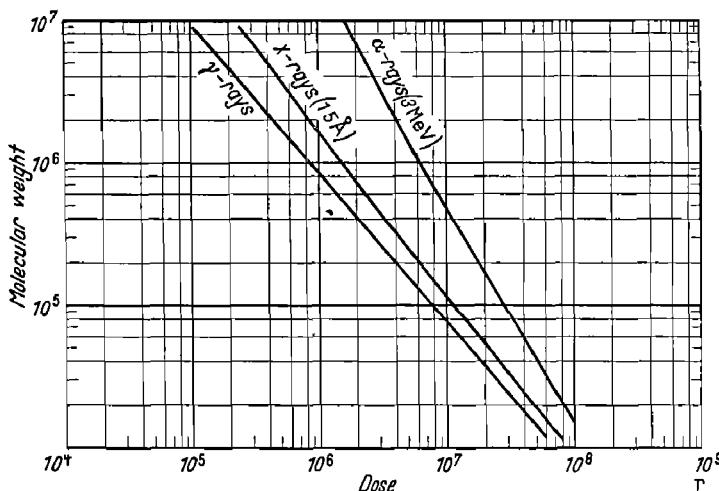
In principle, the calculation of the target size is not difficult. One of the simplest methods, which is applicable for low ion density radiation, is to assume that each ionization occurs singly and at random. The 37 per cent dose in  $r(D_0)$  produces an average of

---

\* In this chapter the following rather arbitrary definitions will be used for *in vivo* and *in vitro* so as to have a convenient notation. *In vivo* is used for experiments on intact animals, plants and micro-organisms. *In vitro* is used for experiments on purely synthetic systems, as well as on enzymes, and virus (including phage) outside their host both, when irradiated dry and when dissolved in water or in broth.

### THE TARGET THEORY

one ionization per target volume  $V$ , which is then obtained directly from the relationship  $V = 0.7/\rho D_0$  in  $\mu^3$  where  $\rho$  is the density of the material irradiated. This simple calculation will give very approximate values only since ionizations do not occur singly, but in clusters, nor are they randomly distributed, but are formed along tracks. These factors can be corrected for, but when the size of the target is greater than the distance separating consecutive ionizations the whole basis of this simple relationship fails. Many attempts have been made to refine the computation and the most successful is probably that due to LEA<sup>4</sup>, which is fully described in his monograph. It is known as the 'associated volume method' and applies to targets of all sizes and for radiations differing in ion density. The results are shown graphically in *Figure 6*.



*Figure 6. Calculation of target size (expressed as molecular weight and diameter) for a nucleoprotein of density 1.3 (see p. 11) from the 37 per cent dose for three different radiations: (a) x-rays (average quantum energy 8.2 keV); (b)  $\alpha$ -rays from radium (average quantum energy 830 keV); (c)  $\gamma$ -rays (average quantum energy 3 MeV.) This applies to single-hit inactivation process and a spherical target; data taken from LEA<sup>4</sup>*

**Effect of different radiation**—A crucial test for the single ionization theory is that the target volume calculated shall be the same for different radiations. With many viruses and phage this is, in fact, the case, and the data from many sources has been collected by LEA<sup>4</sup>. For some of the large viruses, however, this requirement is not met. The targets shown in *Table II* were all obtained by the associated volume method; the agreement for the smallest virus is excellent, the differences for the intermediate virus are probably not significant, while for the large vaccinia virus the single-hit target theory does not apply.

## DIRECT AND INDIRECT ACTION

Table II. Comparison of Target Size calculated by LEA<sup>4</sup> with Microscopic Dimensions of three Viruses

Virus	Target diameter calculated from inactivation dose			Microscopic size (unhydrated)
	$\gamma$ -rays	1.5 Å x-rays	4 MeV $\alpha$ -rays	
phage S13	15.5	15.9	16.3	16 m $\mu$
staph phage K	31	40	50	64 m $\mu$
Vaccinia virus	31	41	70	200 m $\mu$

A possible interpretation is that the larger organism contains a number of separate and discrete sensitive areas (*i.e.* targets), a hit in any one of which leads to inactivation. The inactivation dose with radiations of low specific ionization gives the total volume of all the targets, while the size of each individual target can be found from the *relative biological effect* (RBE), the ratio of the effectiveness of radiations having different ion densities along their tracks (*i.e.* having different *rates of loss of energy*, RLE). When the single-hit theory applies the RBE decreases with increasing RLE. In the case of the vaccinia virus the RBE does not fall as rapidly with RLE as demanded by theory and the target size calculated decreases with increase in RLE of the radiation used.

However, if the assumption has to be made that the organism contains several targets the whole theory becomes much less convincing, since a number of arbitrary parameters are now available for fitting the data. LEA<sup>4</sup> obtains consistent values for vaccinia virus by assuming each particle to have 100 different targets, each of 60 Å diameter, and that the ionization in any one of these leads to inactivation of the whole virus.

The observed RBE of different radiation could also be explained if more than one hit is required to inactivate the organism\*, but this cannot be the case here since the inactivation/dose curve would then have to be sigmoid. The fact that it is exponential indicates a one-hit mechanism. In all these discussions it has been tacitly assumed that the specific ionization RLE of the radiations is the only factor concerned in determining the RBE. The possibility that the size and charge of the ionizing particle may by itself influence the result has been rejected (*e.g.* when comparing  $\beta$ - and  $\alpha$ -radiations the particles concerned are of opposite charge and differ in weight by a

\* If several ionizations have to take place within a small target the RBE will at first *increase* with RLE and, when an optimum value has been reached, fall again (see p. 55).

### THE TARGET THEORY

factor of 7000). Since the specific ionization depends on the velocity of the particle (see p. 21) it is possible to obtain heavy particles with the same RLE as electrons of medium energy. TOBIAS<sup>11</sup> found that the effect on yeast was the same for 200 kV x-rays and 190 MeV deuterons, which have the same average specific ionization. This experiment provides experimental proof that the RBE is determined entirely by the RLE (see *Figure 7*) of the ionizing particle\*.

Finally, it must be emphasized that a fall in RBE with RLE also occurs if the action is indirect, since many more radicals are lost by recombination at high RLE when they are formed close together in the track. In general, the yield of chemical reactions in aqueous solution induced by  $\alpha$ -rays is only 5 to 20 per cent that produced by

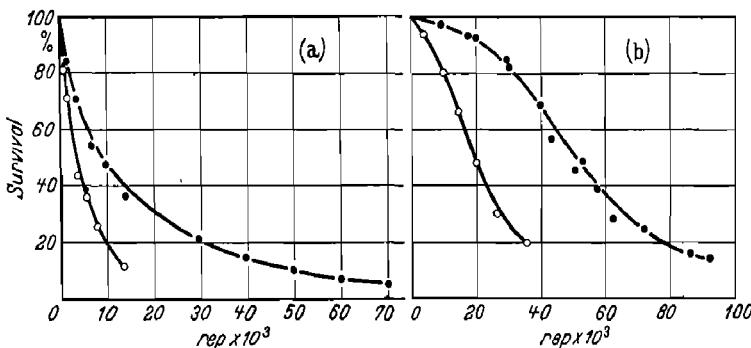


Figure 7. Survival of yeast cells as a function of dose after irradiation with 200 kV x-rays, 190 MeV deuterons and  $\alpha$ -particles from polonium. The specific ionization produced by 200 kV x-rays and 190 MeV deuterons is the same and their biological effects are indistinguishable<sup>11</sup>

- (a) For haploid cells: ● 200 kV x-rays; ○ Po  $\alpha$ -particles; ● 190 MeV deuterons
- (b) For diploid cells; ● 200 kV x-rays; ○ Po  $\alpha$ -particles; ● 190 MeV deuterons

hard x-rays. The influence of specific ionization RLE on chemical reaction in water is discussed in detail in Chapter 3.

*Target size and molecular dimension*—The single-hit target theory, as developed by Lea, does not contain the assumption that the target size is the same as the geometrical dimensions of the biological material. In fact Lea has stressed, that by revealing the size and possibly the number of radio-sensitive sites within an organism the application of this theory to radiation data can reveal biologically important information not otherwise obtainable. None the less for

---

\* This is an over-simplification since the size and charge of the particle influence  $\delta$ -ray production, and where these play an important part the effectiveness of a heavy particle and an electron having the same RLE may be different.

## DIRECT AND INDIRECT ACTION

the smaller viruses, and in particular for enzymes and other biologically active macromolecules, the target size is closely similar to the volume (or molecular weight) of the biological material as determined by direct physical methods. In recent years this technique has provided, in the hands of Ernest POLLARD<sup>1</sup> and his associates, information of the greatest importance concerning the size and organization of biologically active molecules. For nucleic acids, the size of which cannot be determined unambiguously by physico-chemical methods (see p. 144), POLLARD<sup>10</sup> was able to obtain a reasonable estimate by deuteron bombardment. By using radiations having widely different specific ionizations (*e.g.* high energy electron and deuteron beams) the shape of the target can also be calculated.

The data for phage and viruses have been analysed by LEA<sup>4</sup>. The agreement between the actual sizes and those calculated from the target theory by the associated volume method is highly impressive for the smaller organism and is clearly not fortuitous. It is particularly remarkable that in no case is the target size significantly greater than the real size. For enzymes and other biologically active macromolecules the target size (expressed as molecular weight) is generally of the same order as that determined directly (see *Table III*). Occasionally, as in the inactivation of antigenic material and the enzyme catalase, the target volume represents a

*Table III. Comparison of the Target Size of some Macromolecules obtained from Inactivation by Ionizing Radiations with the Molecular weight determined by Physico-chemical Methods*

Material used	Radiation used	Criterion for inactivation	Target size	Molecular weight
Haemocyanin	$\alpha$ -rays	<i>Splitting of molecule into half</i>	$9 \times 10^6$	$9 \times 10^6$ (15)
Ribonuclease	x-rays	<i>Enzymatic activity</i>	12,200	12,000 (8)
Trypsin	Deuterons	" "	30,600	36,000 (16)
Pepsin	"	" "	39,000	36,000 (16)
Insulin	"	<i>Biological assay</i>	23,000	(multiple of (21) 12,000)
Tobacco ringspot virus	x-rays	" "	$2.3 \times 10^6$	$3.4 \times 10^6$ (8)
Desoxyribonuclease	Deuterons	<i>Enzymatic activity</i>	62,000	63,000 (17)
DNA (transforming factor)	"	<i>Pneumococcus transformation</i>	$6 \times 10^6$	$6 \times 10^6$ (10,19)
DNA (from thymus gland)	"	<i>Ability to be digested by desoxyribonuclease</i>	12,000	$6 \times 10^6$ (18)
Bovine serum albumin	"	<i>Antibody-antigen reaction</i>	4000	70,000 (20)

### THE TARGET THEORY

small fraction only of the whole molecule. A possible, though not the only explanation, is that the biological specificity resides in one part of the molecule only (*i.e.* the dose required to inactivate a molecule is greater than if the whole molecule was vulnerable).

For many of the biological materials listed in *Table III* the inactivation dose has been determined with different radiations and with the two exceptions listed in *Table II* a consistent estimate has been obtained for the target size. This has been interpreted by Lea as strong evidence for the view that one ionization in the target is sufficient for inactivation. The point has been raised that since ionizations occur in clusters (see p. 22) it is the cluster and not the single ionization which is effective. This has been countered by LEA<sup>22</sup>, who showed that  $\gamma$ -rays were more effective than x-rays while the reverse would be expected if small clusters were necessary.

In the 'associated volume method' treatment of the target theory, the inherent assumptions are that the target is spherical, that each ionization produces an inactivation and that there is sharp boundary to the target. At first sight it may appear that none of these conditions apply in practice and the excellent agreement observed for the viruses may be due to a mutual cancellation of errors. LEA<sup>4</sup>, however, points out that this simple model seems to be close to reality for the cases studied by him (*i.e.* phage and viruses), since deviations from any one of these assumptions would make the target size calculated from radiations of high RLE higher than that calculated from low RLE. The consistent estimates for the target size of phage and viruses obtained with different radiations cannot, therefore, be ascribed to a cancelling of errors and appears to justify the assumptions made.

The view is widely held that the target size represents a volume which is very sensitive to chemical change, so that an ionization anywhere within it leads to inactivation. Consequently for many materials the sensitive volume extends over the whole organism so that any chemical change resulting from an ionization should lead to loss of biological activity. These conclusions are at variance with current radio-chemical researches which show that in solids, factors are operative (see p. 42) which often reduce the yield per ionization to less than one. Consequently, an ionization may occasionally fail to bring about any chemical change while the target theory still demands that it produces an inactivation. Also many enzymes and small viruses, materials for which the whole molecule is the target, have been chemically modified without loss of activity. This indicates that there are groups within these macromolecules which

#### DIRECT AND INDIRECT ACTION

are not important, yet the target theory demands that every part of the molecule is equally vulnerable.

The difficulty, that a single hit in the target should not always produce inactivation, can probably be resolved by taking excitation into consideration as well. LEA<sup>4</sup> has expressed the opinion that excitation is a good deal less efficient than ionization in the decomposition of macromolecules. This view, however, cannot be maintained in the light of recent experiments with synthetic polymers (see p. 126). Thus ALEXANDER and CHARLESBY<sup>23</sup> have shown that when polyisobutylene is irradiated only 17 eV are required to break the main chain at one point. Assuming that 32.5 eV is required per ionization (the whole basis of the target theory) then in this case excitation must have contributed to the reaction. Moreover, u.v. light<sup>24</sup> has been shown to inactivate—admittedly with a low quantum yield—many of the viruses studied with ionizing radiation, so that it is clear that an excitation at the right site can produce the same result as an ionization. The number of excitations almost certainly exceeds the number of ionizations, possibly by as much as a factor of three; on this basis the one-hit theory would have to be redefined as follows: inactivation occurs when one ionization and an unknown number of excitations, neither of which need necessarily result in a chemical change, are produced in the target area. This clearly is not a useful concept, and since no direct information is available for solids concerning ionizations produced by ionizing radiation it would appear that calculations necessary for establishing the number of chemical changes and the size of the sensitive area cannot be made.

The recent discovery that the target size calculated from deuteron bombardment for some enzymes<sup>12</sup> increases with temperature (*i.e.* the dose required for inactivation decreases) also indicates that the basic concepts of the target theory require revision. *Figure 8* illustrates this effect for catalase. A possible explanation which has been advanced by POLLARD<sup>1</sup> is that at higher temperatures the bonds, which have to be broken for inactivation to occur, are weaker so that they can be severed both by ionizations and excitations. At low temperatures only ionizations can bring about the required reaction.

*Energy transfer*—As already pointed out, the successes of the target theory in giving the size of biological materials cannot be dismissed as fortuitous, and most of the difficulties can be resolved by taking the possibility of energy transfer into consideration. Essentially this implies that the chemical change brought about by ionizing radiations need not occur at the site where the energy is absorbed

### THE TARGET THEORY

(either by ionization or excitation) but that it can be transferred over considerable distances. Since the experiments of Svedberg and Brohult on the splitting of the haemocyanin molecule into discrete and well-defined sub-units by the passage of a single  $\alpha$ -particle through the giant molecule (see p. 135), further evidence has been found of energy transfer processes in macromolecular systems<sup>23</sup> (see p. 127). The application to the target theory of the principle that chemical changes induced by exposure to ionizing radiations need not occur at random in solids, but that the energy absorbed can become localized in certain chemical groups or molecules, would appear to be justified. According to this view the final chemical changes may be localized, even though the original uptake of energy

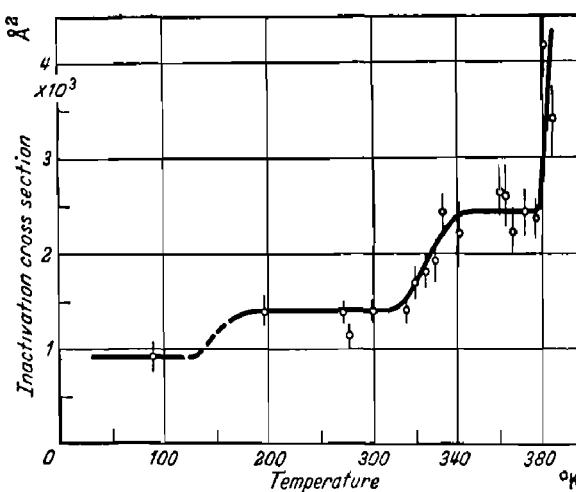


Figure 8. Variation of target size of catalase (expressed as apparent cross-section) with temperature. The curve shows that the enzyme becomes more radio-sensitive as the temperature is increased<sup>12</sup>

by a macromolecule is uniform. The target is then seen to represent the volume over which energy can be transferred to a vulnerable point, where it brings about a chemical reaction leading to inactivation. The fact that the target sizes show the best agreement with physical dimensions for viruses having a diameter of less than 200 Å indicates that energy transfer in protein (and nucleoprotein) molecules can occur over these distances. The influence of temperature on the sensitivity of some enzymes can also be interpreted on the basis that energy transfer is favoured (*i.e.* the localization of energy is more effective) at higher temperatures<sup>78</sup>,

The theory that the whole of the target volume is not equally

#### DIRECT AND INDIRECT ACTION

radio-sensitive also explains the much lower efficiency of indirect as compared with direct action for the inactivation of viruses. On the original target theory this difference is difficult to understand<sup>5</sup> since any reaction is postulated to result in loss of activity. The free radicals from water are highly reactive and can bring about changes similar to those produced by direct action, and in many chemical reactions the indirect reaction is more efficient than the direct. High reactivity, however, enables the radicals to interact readily with many chemical groups, and with a virus they may react at a number of points so that many free radicals may be wasted in bringing about reactions which do not result in loss of biological activity: therefore, reaction at vulnerable points may occur more readily by direct action when energy transfer can take place. The reaction of free radicals will not be completely random, and this is illustrated by the wide variation in the effectiveness of different compounds as protective agents. Steric factors could also contribute to the low efficiency of indirect action, since the vulnerable groupings may be in the centre of the molecule, which are relatively inaccessible to free radicals, but can receive energy by a transfer process.

#### APPLICATION OF THE TARGET THEORY TO RADIATION EFFECTS PRODUCED *IN VIVO*

Before Dale's experiments<sup>3</sup> with enzymes had clearly established that inactivation of biological materials could occur by indirect action, biological radiation data was often analysed in terms of the target theory. A reaction was usually assumed to be of the single-hit target type if (i) it was independent of the dose rate, (ii) it gave an exponential inactivation/dose curve, and (iii) showed the expected relative biological effectiveness (RBE) for different radiations. When these requirements were not followed the results were often fitted to a multi-hit model. However, all the criteria mentioned for a 'one-hit' target model apply also to the simplest cases of indirect action where the inactivated material competes for the free radicals (see p. 50). The more complex (*i.e.* sigmoid) inactivation/dose curves which are fitted to multi-hit target models can also be obtained by indirect action when reasonable assumptions concerning diffusion in heterogeneous systems are made. Before the target theory in any form can be applied it is necessary, therefore, to prove that the inactivation or other change was produced directly and not by free radicals. One way to be certain of this is to irradiate the biological material dry when no indirect action can occur and

## TARGET THEORY AND RADIATION EFFECTS *IN VIVO*

many of the experiments discussed in the previous section were carried out in this way. Alternatively, if the irradiation is carried out in the presence of water the most decisive test is to irradiate the material at different concentrations. If the percentage inactivation is independent of concentration (or the number of molecules changed proportional to concentration) then the action may be assumed to be direct.

Experiments of this type cannot, however, be carried out readily *in vivo* and it is extremely difficult to determine by which mechanism the important radiobiological effects, such as gene mutation, chromosome breakage, mitosis arrest and killing, are brought about. Contrary to earlier views, present indications are that indirect action plays an important part in all these processes.

*Irradiation of frozen material*—Svedberg and Brohult (see p. 135) showed that freezing of solutions reduced the radiation effect of a given dose if this occurs by indirect action by eliminating diffusion processes. Temperature changes did not influence the splitting of the haemocyanin molecule which was brought about by direct action. If other processes (*e.g.* excitations) intervene, as was found for the irradiation of certain enzymes (*cf. Figure 8*), an observed temperature dependence may not necessarily imply that the radiation process is indirect. If the effectiveness is the same in the frozen as in the liquid state the process cannot occur via free radicals, and unless an energy transfer process occurs the action must be direct. Bearing these reservations in mind, RAJEWSKY's<sup>25</sup> finding that the growth inhibition of seeds and pollen by x-rays is 75 per cent less when carried out at the temperature of liquid air than at room temperature argues strongly against direct action in this case. LEA *et al.*<sup>26</sup> found that bacterial spores were killed equally effectively at -20° C and +50° C, suggesting that the process is due to direct action. A most puzzling feature is that  $\alpha$ -rays are more effective than x-rays, while the survival curve is exponential. The target theory can, therefore, not be applied and the inactivation may have to be interpreted by another model, which has been put forward by Gray for chromosome breakage (see p. 73).

*The effect of oxygen and of protective agents*—The low temperature test can only be applied with very specialized material, and probably the most useful criterion for direct action generally is to determine if added substances (*i.e.* protective agents) or a change in oxygen pressure in the system influence the effect. While a negative result does not prove direct action as a protective agent may not penetrate to the site of action, a positive result indicates indirect action.

#### DIRECT AND INDIRECT ACTION

This test must be used with discretion since in biological systems the radiations initiate a chemical change, but the effect actually observed is several stages removed from this first process. Even if the action of the radiation is direct, added materials or differences in oxygen concentration may influence the change measured by interfering with the intermediate steps, such as recombination and reconstitution processes. If the protective agent has pharmaco-dynamic properties the possibility that it changes the response of the organism must be guarded against. For instance, by changing a metabolic pathway, inactivation of a given enzyme system may no longer prove fatal. The fact that protection has occurred does not then establish that the radiation induced reaction was necessarily indirect.

By conducting suitable control experiments most of these objections can be ruled out and when protection by added substances, coupled with potentiation by oxygen, is observed, indirect action can be presumed to be important. Oxygen converts one of the free radicals formed in water into a more powerful oxidizing entity (see p. 104) and this is the reason why it potentiates indirect action (see Chapter 8). Potentiation of direct processes by oxygen has not been observed in biological systems. Thus there is no oxygen effect for the irradiation of frozen bacteria<sup>27</sup> while in suspension when the action is indirect there is a pronounced potentiation by oxygen. Similarly the sensitivity of dry barley seeds<sup>28</sup> and the irradiation of viruses in broth<sup>29</sup> (*i.e.* so heavily protected that indirect action is suppressed) is not affected by changes in oxygen pressure. The possibility that oxygen potentiates direct action cannot be wholly excluded<sup>78</sup>. For example direct action may produce a labile point in a macromolecule which on combination with oxygen leads to a fracture initiating a biological lesion.

*Mutations*—The production of gene mutation has often been interpreted in terms of the target theory and as early as 1931 BLACKWOOD<sup>30</sup> calculated the size of a gene from radiation data, and this approach was extended by TIMOFEEFF-RESSOVSKY<sup>31</sup>; in his monograph, *Das Treffer Principe in der Biologie*, he applied Crowther's method of analysis<sup>9</sup> widely, and with much less discrimination than was indicated in the original publication. Many of the deductions were severely criticized, notably by MULLER<sup>32</sup>, but more recently the new experiments, and above all, the refined method of analysis of CATCHESIDE and LEA<sup>33</sup> brought new support for the target hypothesis.

Gene mutation was claimed by LEA<sup>4</sup> to be the most clearly established example of a radiation effect *in vivo* produced by the single-hit target mechanism, since the number of mutations produced in a

#### TARGET THEORY AND RADIATION EFFECTS *IN VIVO*

population is (i) proportional to the dose (the first part of an exponential curve is a straight line); (ii) independent of dose rate; and (iii) decreases with increasing specific ionization of the radiation used. Although these are the principal tests by which a single-hit action can be recognized, they are only valid if the action has been shown to be direct, as the same relationship would also apply to simple indirect action. Decisive evidence on this point is difficult to obtain, but the observation by HOLLAENDER and his colleagues<sup>34</sup> that the oxygen concentration markedly influences the number of gene mutation in *Drosophila* and *Tradescantia* (see Chapter 8) would favour an indirect mechanism. There is also evidence (see Chapter 14) that certain chemical substances can protect against the genetic effects of x-rays.

The observed fall in RBE with increasing RLE, which is essential if a single-hit interpretation is to be maintained, is illusory (*cf.* Table IV). MULLER and VALENCIA<sup>35</sup> were able to prove that the smaller effectiveness of neutrons, when compared with x-rays, is not real but is the result of a number of reactions on neighbouring genes, which cannot readily be detected separately.

Another objection to the interpretation of Catcheside and Lea is that the size of the target volume calculated (and this is identified with the size of a gene) corresponds to a macromolecule of molecular weight between  $10^4$  and  $10^5$ . This value is much too small according to Muller, since it implies that only 0.1 per cent of the total mass of the chromosome consists of genetic material, a conclusion which cannot be reconciled with other data. Moreover, there is considerable evidence that deoxyribonucleic acid (DNA) is intimately related to genes, and it is now known (see p. 144) that this material has a molecular weight of the order of many millions (*i.e.* 100 times the target size). Though none of these objections are decisive by themselves in conjunction they represent formidable evidence against the view that gene mutation *in vivo* is the result of a single ionization within a gene.

*Killing of bacteria*—LEA, HAINES and COULSON<sup>36</sup> interpreted the killing of bacteria as a lethal mutation produced by a single ionization within a sensitive volume, which they tentatively identify with chromosomal material. In their own experiments the survival curves were exponential, and the effect was independent of dose rates. However, the mean lethal dose for radiation of different ion densities did not lead to consistent values for the target size and this was interpreted, in terms of a multi-target model in the same way as for the vaccinia virus (see p. 58). For *Escherichia coli* the assumption of 250 targets (called genes) of 120 Å diameter fits the

#### DIRECT AND INDIRECT ACTION

experimental data, but this agreement is not impressive since two parameters can be chosen at will to fit the experimental points.

Although LEA *et al.*<sup>36</sup> obtained exponential curves many other workers had found sigmoid survival curves, and these were dismissed by LEA<sup>4</sup> as being brought about by bad experimental technique. Recent work, notably by HOLLÄENDER and his colleagues<sup>37</sup> has shown beyond doubt that all types of survival curves can be obtained, ranging from exponential to highly pronounced sigmoid curves depending entirely upon the condition of the experiment. It is possible to interpret the sigmoid curves in terms of the target theory if

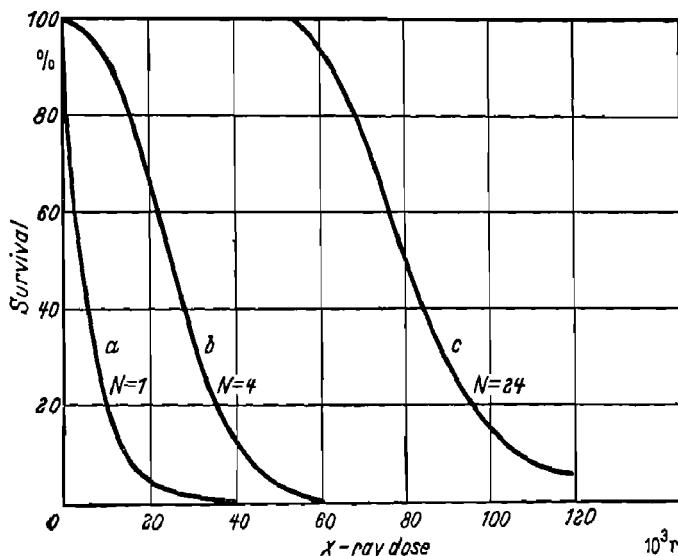


Figure 9. Survival curves of bacteria cultured under different conditions. These results fit the relationships required for the multi-target theory, but the number of targets ( $N$ ) which have to be inactivated to kill an organism apparently varies with culture conditions;  $a$  = aerobic broth culture exposed in oxygen;  $b$  = aerobic or anaerobic glucose broth culture exposed in oxygen;  $c$  = anaerobic glucose broth culture exposed in nitrogen

more than one hit is necessary for inactivation. The number of hits is given by the shape of the curve (see Figure 5). This view, however, is quite untenable since it would require that the number of targets can be changed at will by adjustment of the culture conditions (see Figure 9). Unambiguous evidence that the killing of bacteria by ionizing radiations is an indirect effect and that the target theory is not applicable was produced by HOLLÄENDER's group<sup>38</sup>, who showed that both anoxia and added chemicals exercise a protective effect, which under optimum conditions can be as high as 98 per cent.

## TARGET THEORY AND RADIATION EFFECTS *IN VIVO*

The finding<sup>59</sup> that recovery of irradiated bacteria (*Escherichia coli*) after irradiation can be obtained by incubating at sub-optimal temperatures between 6 to 30° C, further emphasizes the many steps which intervene between the minute primary effect and the observed end result. The surviving fraction is greatest when the organisms are stored for 12 hours at 18° C in a suitable medium before being cultivated at the normal temperature of 37° C. This storage produces an effect equivalent to a reduction in dose of 30 per cent. There are indications that many of the primary lesions are repaired even under normal conditions and thereby escape detection. Some of these are revealed if the cell is exposed before or after irradiation<sup>62</sup> to infra-red radiations which presumably interfere with the repair mechanism.

An example related to the irradiation of bacteria is the killing of yeast<sup>11</sup> shown in *Figure 7*. The survival curve of the haploid cells is exponential, while that of diploid cells is sigmoid. This is exactly the behaviour which would be predicted by the target theory; haploid cells having only one set of chromosomes present one target, while in diploid cells with double the number of chromosomes two targets have to be hit if the cell is to be inactivated. The observation that for both types of cell  $\alpha$ -particles are more than twice as effective as radiations of lower specific ionization cannot be reconciled with the target theory. This behaviour could be interpreted on a multi-hit mechanism for diploid cells, but is quite inconsistent with a single-hit target for the haploid cell. Probably this complex behaviour, found for the killing of cells, can best be explained on the basis of the destruction of a vital structure which requires a minimum local concentration of free radicals (see Chapter 6).

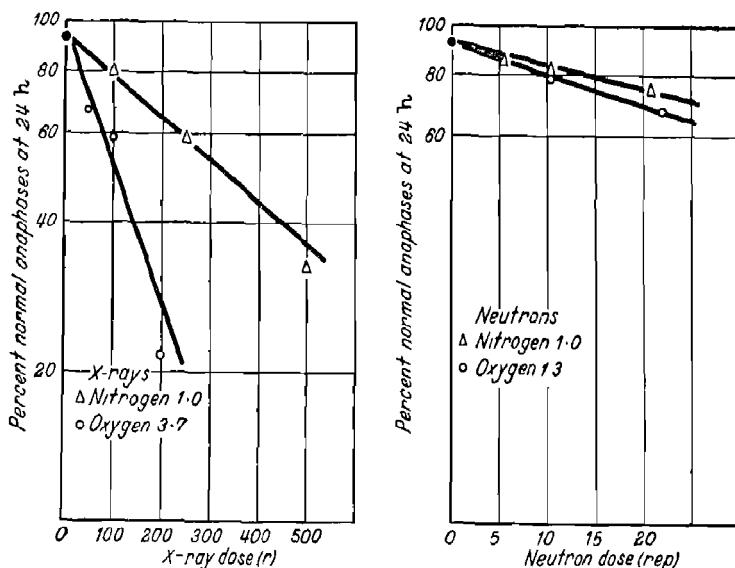
*Chromosome aberrations*—The target theory in a modified form has also been applied to the production of chromosome aberrations (see Chapter 5). Interpretation here is exceptionally difficult, since many stages—none of them understood—intervene between the observed change and the radiation effect. At first sight it would appear as if all the evidence was against the target theory, since the dose/effect curve is only rarely exponential, the number of aberrations per given dose increases sharply with increase in dose rate as well as temperature and finally densely ionizing radiations are more effective than x-rays.

Undaunted by these difficulties LEA and CATCHESIDE<sup>39, 4</sup> have fitted the data to the hypothesis that each break is caused by the passage of a single ionizing particle through the breakage point by postulating: (i) that the majority of the breaks can rejoin by restitution or exchange (see Chapter 5) for several minutes after the break

#### DIRECT AND INDIRECT ACTION

has occurred and are not, therefore, detected; (ii) that the number of ionizations which must be produced within a chromosome to give a break is of the order of 15 to 20.

From the first assumption it follows that the number of aberrations *seen in the experiment* will increase with increasing dose rate. The second postulate provides that the RBE increases with RLE. Thus the average in density along high energy electron tracks from  $\gamma$ -rays or hard x-rays is too low to produce the number of ionizations necessary for a break within the cross-section of a chromosome and only the tail end of an electron track will be effective. This means



*Figure 10. Survival of mouse ascites tumours after irradiation with x-rays and neutrons. The effect of removing oxygen is much greater with x-rays than with neutrons<sup>49</sup>*

that the probability of a break being produced by an ionizing particle passing through a chromosome is approximately one for protons (or neutrons) and for  $\alpha$ -rays of normal energy, but less than one for radiations of low specific ionization. In agreement with this theory 250 kV x-rays were found to be<sup>60</sup> twice as effective as  $^{60}\text{Co}$   $\gamma$ -rays in producing chromosome aberrations in *Tradescantia* pollen. Using the mouse ascites tumour GRAY and CONGER<sup>61</sup> also found that the densely ionizing radiations from neutrons were about three times as effective as 190 kV x-rays in producing chromosome abnormalities at anaphase (see *Figure 10*).

This interpretation for the production of chromosome aberrations has been generally successful in representing much of the

#### TARGET THEORY AND RADIATION EFFECTS *IN VIVO*

experimental data quantitatively, but new facts have shown up its inadequacy. KOTVAL and GRAY<sup>40</sup> found  $\alpha$ -particles to be more effective than neutrons (*i.e.* ejected protons). On Catcheside and Lea's hypothesis the contrary result would be expected because the ion density along a proton track is already sufficient to produce a break (*i.e.* to produce 20 ionizations within a chromosome);  $\alpha$ -radiation should be less effective since many more ionizations will be wasted.

More important still is the finding that the concentration of oxygen exerts a pronounced influence (see Chapter 8) on the number of aberrations observed after irradiation with x-rays in all materials which have so far been examined<sup>34, 41, 42, 61</sup>. The possibility that oxygen does not change the radiation effect, but reduces the recovery process thereby making more of the breaks visible has been eliminated by careful experiments<sup>41</sup>. The most plausible explanation seems to be that in the presence of oxygen a substance is formed which increases the number of aberrations. The findings of THODAY and READ<sup>42</sup> that the protective effect of anoxia was much smaller with  $\alpha$ - than for x-rays supports this view since the influence of oxygen in many radio-chemical experiments is much greater with x- than with  $\alpha$ -rays (see p. 106).

By comparing the frequency of dominant lethal mutation in *Drosophila* with chromosome aberrations BAKER and von HALLE<sup>43</sup> reached a rather different conclusion. They believe that the potentiating effect of oxygen was not due to the fact that in the presence of oxygen more breaks occurred, but that the type of break produced in oxygen is less liable to reconstitute. If this is the mechanism then the oxygen effect could be reconciled with both direct and indirect action. But as there is no oxygen effect with radiations of high specific ionization, the possibility that oxygen combines with a molecule which has been ionized directly and thereby changes its reactivity\*, is ruled out.

Consideration of all the evidence makes it unlikely that direct action plays a predominant part in the breakage of chromosomes. But one of the concepts proposed by Lea, namely that a certain minimum number of reactions must occur simultaneously before a break is seen, has to be retained even if the process is indirect.

---

\* This behaviour was found in a purely synthetic system (see p. 130); when concentrated solutions of polyvinyl alcohol are irradiated, cross-linking occurs only in the absence of oxygen since the latter combines with the polymer radical produced by irradiation and renders it incapable of cross-linking.

## DIRECT AND INDIRECT ACTION

### INDIRECT ACTION *IN VIVO*

In the foregoing section it was shown that the target theory is not applicable, at least in its simple form, to most of the *in vivo* changes produced by ionizing radiations.

*Poison theory*—The possibility that the action of radiation is due to the formation of a stable poisonous material is generally dismissed and only very few experiments have been carried out to test the point. Certain nutrient media for bacteria cultures are rendered toxic by irradiation<sup>44</sup> though only with doses considerably greater than those required to kill the bacteria. The viability<sup>45</sup> and rate of respiration<sup>46</sup> of sea-urchin sperms is reduced when sea water is heavily irradiated, but if the organisms were present during the irradiation the effect was much more marked. The possibility remains that the poison must be produced *in situ*, but a sigmoid survival curve typical of cumulative action would then be expected since no killing would occur in the early stages of irradiation when the concentration of the poison is low; if the resistance of the organism is fairly uniform a sharp drop in survival should occur when the cells have been exposed for a time sufficient to produce a lethal quantity of poison. Exponential curves, frequently observed with ionizing radiation, could only be interpreted on the poison hypothesis by assuming a most unusual distribution of resistance<sup>4</sup>. Quantitatively the poison theory is not impossible. Thus if the poison has a molecular weight of 1000 and is produced with an ionic yield of one, a lethal whole body irradiation of 500 r would produce about 50 mg of poison in a man. This would mean that the substance produced would have to be as toxic as strychnine, but much less so than many toxins.

*Influence of hydration*—Living systems which survive desiccation, or which like seeds become desiccated during a stage of their development, are in general very much less sensitive to radiations than organisms which contain a high proportion of water<sup>50-55</sup>. However, in the case of seeds the retention of water is not the only factor. LAMBERT<sup>56</sup> observed that the radio resistance of peas (*Pisum sativum*) —initially 5 to 10,000 r—increased during the first six to twelve hours after hydration (water content 30 per cent) but then fell rapidly to reach a minimum after 24 hours of about 2500 r. During hydration of the pea, FIRKET and colleagues<sup>57, 58</sup> found in 1929 that the number of SH groups and the amount of ascorbic acid was greatly increased<sup>57, 58</sup> and this may be the cause for the initial increase in radio resistance. These observations all focus attention on the role played by water in the biological effects of radiation.

#### INDIRECT ACTION *IN VIVO*

*Role of free radicals produced in water*—GRAY<sup>47</sup> was the first to modify the target theory to include indirect action by defining the primary act as ‘the dissipation of energy within a strictly localized region without regard as to whether the energy is dissipated within the biological structure or in the immediate surrounding medium’. This definition removes the concept of a target and a radiation effect is called *monotopic* or *ditopic* depending on whether an ionization has to occur in one or in two strictly localized regions. This more generalized view is a development of the target theory, which in common with all theories in a rapidly growing field, has to be modified in the light of new data.

The view that the free radicals produced in water contribute significantly to many of the effects of radiation *in vivo* offers the most consistent interpretation of the different experiments, although decisive proof is lacking for the more complex phenomena. This conclusion is surprising in view of the virus inactivation studies which occur by a direct mechanism even in dilute solution (see p. 53). If gene mutation, killing of bacteria and breakage of chromosomes are produced indirectly the affinity of the free radicals for the macromolecules involved must be much higher than for viruses *in vitro*. There is no justification on chemical grounds for such an assumption and the hiatus between the *in vitro* and the *in vivo* experiments remains to be resolved.

One of the beauties of the simple target theory is that it is immediately amenable to quantitative treatment. The problem at the present time is to analyse indirect actions in quantitative terms. Essentially the problem is to calculate the probability of a free radical, produced in a given volume, reacting with a vital macromolecule in such a way as to render it inactive. Apart from the purely formal difficulties of dealing with diffusion processes in gels, fundamental data concerning the lifetime and diffusion coefficients of free radicals are not available. Nor is the radio-chemistry of water (see Chapter 3) sufficiently developed to provide an unambiguous answer as to the nature of the radicals produced, their reactivities and their relative points of formation in relation to the track of the ionizing particle. In addition the biological material cannot be considered as homogeneous so that diffusion through different phases and across phase boundaries has to be considered.

ZIRKLE and TOBIAS<sup>48, 77</sup> by making suitable simplifying assumptions have obtained a general solution which can be applied to biological data. Both exponential and sigmoid survival curves as well as differences of the RBE with ion density can be obtained by these treatments. The fact that the biological data can be fitted

#### DIRECT AND INDIRECT ACTION

does not constitute a real test for the assumptions made, since the number of parameters, which are chosen arbitrarily, is such as to make it possible to fit almost any data. At present it is probably idle to expect to be able to treat indirect action *in vivo* quantitatively, although a general qualitative pattern is becoming apparent.

*Single- and multiple-hit effects in terms of indirect action*—If only a single reaction by a free radical with a vital centre is required, the effect studied will show an exponential relationship with dose and be independent of dose rate. The RBE will decrease as the specific ionization is increased because the local concentration of the free radicals is higher and more will be wasted by recombination. This aspect is dealt with in detail in the following chapter. In all these respects simple indirect action is qualitatively similar to a single-hit target mechanism and processes, such as gene mutations, can be treated in terms of a reaction with a free radical.

In principle at least, biological systems showing sigmoid survival curves can be interpreted by analogy with the target theory as processes requiring more than one reaction within a sensitive centre. From the shape of the sigmoid curve it is not possible, as in the case of direct ionizations, to deduce the number of reactions required. Because of the importance of diffusion the character of the curve will vary with changing extraneous conditions, and this has in fact been found for the killing of bacteria (see *Figure 9*).

*Indirect action produced by radiations of high specific ionization*—For many radiobiological effects including breaking of chromosomes the RBE increases with specific ionization (see *Table IV*), the same fundamental assumption has to be made for indirect as for direct action, namely that a certain number of radicals must react simultaneously with the biological structure. In other words the biological structure must be exposed to a high concentration of radicals\*. This condition is realized when a densely ionizing particle passes close to or actually through (*i.e.* target theory) the biological structure. Obviously this kind of reaction is required when an organized structure, such as chromosomes or cell membranes, has to be physically damaged. A single chemical reaction can interfere with synthetic processes by inactivating an enzyme or gene, but it cannot disrupt an already existing morphological struc-

---

\* In the tracks of particles having a high specific ionization recombination of the radicals formed in water occurs extremely rapidly and hydrogen peroxide is formed in high local concentration. It is possible that the chemical damage is produced in these cases not by the free radicals but by the concentrated hydrogen peroxide. GRAY<sup>49</sup> has compared irradiation by  $\alpha$ -rays with the injection of fine jets of concentrated hydrogen peroxide. For the purpose of the present discussion it is immaterial whether free radicals or hydrogen peroxide are the causative agents.

INDIRECT ACTION *IN VIVO*

*Table IV. The Relative Biological Effectiveness (RBE)\* of Radiations having different Specific Ionizations (i.e. different Rate of Loss of Energy, RLE)*

Biological effect	Radiations used†	Ratio of effectiveness	Reference
<b>CHROMOSOME ABERRATIONS ‡</b>			
Tradescantia microspores . . .	n:x	4.3:1	62
Barley seeds . . .	n:x	12:1	73
Tradescantia bracteata (anthers) .	α:x	15:1	40
Ascites tumours (mouse) irradiated in oxygen . . .	n:x	3:1	61
Ascites tumours (mouse) irradiated in nitrogen . . .	n:x	8:1	61
Vicia faba abnormal anaphases . .	x:n:α	1:6:8	62
", dose to reduce growth rate to 50%	x:n:α	1:6:6	74
<b>LETHAL EFFECTS</b>			
Vicia faba . . .	α:n:x:γ	9.0:8.7:1.5:1	63
Tradescantia microspores . . .	α:n:x:γ	3.8:2.8:1.1:1	4
Barley seeds (L.D. 50) . . .	n:x	11:1	73
Barley plants (50% unaffected) . . .	n:x	94:1	73
Barley seeds (wet) in air . . .	α:n:x	10:8:1	64
", (dry) . . .	α:n:x	1:15:1	65
", (dry) in nitrogen . . .	n:x	60:1	64
Aspergillus terreus spores . . .	n:x	3.2:1	66
Spores of <i>B. mesentericus</i> . . .	α:γ	5:1	4
Escherichia coli . . .	α:x:γ	0.2:0.8:1	4
Drosophila eggs . . .	n:x	1.1:1	75
Grasshopper nymph ovarioles . . .	n:x:γ	19:1.2:1.0	76
Mouse § . . .	n:x:γ	8-10:1:0.5	67
Transplantable mouse tumour . . .	n:γ	9.5:1	72
irradiated <i>in vivo</i> . . .	n:γ	24:1	72
", <i>vitro</i> . . .	n:γ		
<b>MUTAGENIC ACTION</b>			
Drosophila sex-linked lethal mutations . . .	n:x	0.6-0.8:1	68
Drosophila dominant lethal mutations . . .	n:x	2:1	69
(Low dose range) . . .	n:x	7.3:1	70
(High dose range) . . .	n:x	4.8:1	70
Aspergillus niger spores . . .	n:x	0.6:1	66

**INACTIVATION OF VIRUSES AND ENZYMES**

Where this occurs by a single-hit mechanism under conditions where direct action predominates the RBE depends on the size of the target (*e.g.* for material of molecular weight of  $10^6$  the ratio for  $\alpha:x$  is 0.05:1 while for a molecule of weight  $10^5$  the same ratio is 0.4:1). The experimental values for tobacco mosaic virus and phage S13 is about 0.2:1 for the ratio of the efficiencies of  $\alpha:x$  radiations.

\* The RBE is not an absolute value for any particular system, but depends amongst other factors on the temperature, oxygen concentration and often on the dose rate. The RBE  $\alpha:x$  is defined as

$$= \frac{\text{x-ray dose required to produce effect}}{\text{α-ray dose required to produce effect}}$$

† In general experiments using x-rays having energies within the normal therapeutic range of 50 to 400 kV were chosen. The neutrons and α-particles had energies of the order of a few MeV.

‡ The values for chromosome damage are approximate only and the ratios vary with the time interval between irradiation and fixation and with the type of abnormality scored. The results quoted refer either to the total number of breaks or to the total of abnormal anaphases observed.

§ Certain tissues are much more sensitive than others to radiations of high ion density. In spite of the great variation of the RBE (n:x(γ)) for lethal effects all authors<sup>71</sup> are agreed that the value for cataract formation and for sterility are greater than that of the L.D. 50 value.

#### DIRECT AND INDIRECT ACTION

ture. For such a process only tracks having an ion density greater than a certain minimum can be effective at all. With hard radiations only the end of the track will produce a sufficient local concentration of radicals and the large majority of the ionizations will be wasted, since they are too far apart to produce the necessary concentration of radicals. This waste will decrease as the specific ionization increases.

With densely ionizing radiations the whole of the track will be surrounded by a column of radicals, the concentration of which decreases radially. According to this theory the ionizing particle need not traverse the biological object, which will be acted upon so long as it is within the range of the free radical envelope of the track. This represents the significant difference between direct and indirect action for processes such as chromosome breakage, and explains why, contrary to Lea's hypothesis, the RBE is greater for  $\alpha$ -particles than for protons. The distance between the particle track and the chromosome (*i.e.* the amount by which the ionizing particle can 'miss' the target) at which aberrations are still produced increases with increasing specific ionization, since the free radical envelope extends further. Therefore, fewer  $\alpha$ -particles than protons will be wasted by missing the chromosomes altogether. With the limited data available it is not possible to formulate this model quantitatively.

#### REFERENCES

- <sup>1</sup> POLLARD, E. C., *Advanc. biol. med. Phys.*, 1954, **3**, 153
- <sup>2</sup> DALE, W. M., GRAY, L. H. and MEREDITH, W. J., *Phil. Trans.*, 1949, **242A**, 33
- <sup>3</sup> DALE, W. M., *Biochem. J.*, 1942, **36**, 80; *Disc. Faraday Soc.*, 1952, **12**, 293
- <sup>4</sup> LEA, D. E., *Actions of Radiations on Living Cells*, Cambridge Univ. Pr., 1946
- <sup>5</sup> LEA, D. E., *Brit. J. Radiol.*, Suppl. No. 1, 1947, p. 59
- <sup>6</sup> GORDON, S. and BURTON, M., *Disc. Faraday Soc.*, 1952, **12**, 88
- <sup>7</sup> FRICKE, H., HART, E. J. and SMITH, H. P., *J. chem. Phys.*, 1938, **6**, 229
- <sup>8</sup> LEA, D. E. and SMITH, K. M., *Parasitology*, 1940, **32**, 405; 1942, **34**, 227
- <sup>9</sup> CROWTHER, J. A., *Proc. roy. Soc.*, 1924, **B96**, 207
- <sup>10</sup> FLUKE, D., DREW, R. and POLLARD, E. C., *Proc. nat. Acad. Sci., Wash.*, 1952, **38**, 180
- <sup>11</sup> TOBIAS, C. A., *Symposium on Radiobiology*, Wiley, New York, 1952, p. 357
- <sup>12</sup> SETLOW, R. and DOYLE, B., *Arch. Biochem. Biophys.*, 1953, **46**, 46
- <sup>13</sup> ADAMS, W. R. and POLLARD, E. C., *ibid.*, 1952, **36**, 311
- <sup>14</sup> HUBER, W., *Naturwissenschaften*, 1951, **38**, 21
- <sup>15</sup> SVEDBERG, T. and BROHULT, S., *Nature*, 1938, **142**, 830

## REFERENCES

- <sup>16</sup> POLLARD, E. C., BUZZELL, A., JEFFREYS, C. and FORRO, F., *Arch. Biochem. Biophys.*, 1951, **33**, 9
- <sup>17</sup> SMITH, C. L. *ibid.*, 1953, **45**, 83,
- <sup>18</sup> SMITH, C. L., *ibid.*, 1953, **46**, 12
- <sup>19</sup> REICHMANN, M. E., BUNCE, B. H. and DOTY, P., *J. Polym. Sci.*, 1953, **10**, 109
- <sup>20</sup> HUTCHINSON, F., *Arch. Biochem. Biophys.*, 1952, **41**, 317
- <sup>21</sup> SETLOW, R. and DOYLE, B., *ibid.*, 1953, **42**, 83
- <sup>22</sup> LEA, D. E., *Brit. J. Radiol.*, Suppl. No. 1, 1947, p. 39
- <sup>23</sup> ALEXANDER, P. and CHARLESBY, A., *Nature*, 1954, **173**, 578
- <sup>24</sup> McLAREN, A. D., *Advanc. Enzymol.*, 1949, **9**, 75
- <sup>25</sup> RAJEWSKY, B. N., *Brit. J. Radiol.*, 1952, **25**, 550
- <sup>26</sup> LEA, D. E., HAINES, R. B. and COULSON, C. A., *Proc. roy. Soc.*, 1936, **120B**, 47
- <sup>27</sup> HOPPENBROEK, A., private communication
- <sup>28</sup> EHRENBERG and GUSTAFSSON, A., *Acta radiol., Stockh.*, 1954, **41**, 101
- <sup>29</sup> HEWITT, H. B. and READ, J., *Brit. J. Radiol.*, 1950, **23**, 416
- <sup>30</sup> BLACKWOOD, O., 1930, *Phys. Rev.*, 1931, **37**, 1698
- <sup>31</sup> TIMOFÉEFF-RESSOVSKY, N. W., *Das Treffer Prinzip in der Biologie*, Leipzig, 1947
- <sup>32</sup> MULLER, H. J., *Symposium on Radiobiology*, p. 328, Wiley, New York, 1952
- <sup>33</sup> CATCHESIDE, D. G. and LEA, D. E., *J. Genet.*, 1945, **47**, 1 and 25
- <sup>34</sup> HOPPENBROEK, A., BAKER, W. K. and ANDERSON, E. H., *Cold Spring Harb. Symp. quant. Biol.*, 1951, **16**, 315
- <sup>35</sup> MULLER, H. J. and VALENCIA, T., *Genetics*, 1951, **20**, 115
- <sup>36</sup> LEA, D. E., HAINES, R. B. and COULSON, C. A., *Proc. roy. Soc.*, 1937, **123B**, 1
- <sup>37</sup> HOPPENBROEK, A. and STAPLETON, G. E., *Physiol. Rev.*, 1953, **33**, 77
- <sup>38</sup> HOPPENBROEK, A., STAPLETON, G. E. and BILLEN, D., *Trans. Faraday Soc.*, 1953, **49**, 331
- <sup>39</sup> CATCHESIDE, D. G. and LEA, D. E., *J. Genet.*, 1943, **45**, 186
- <sup>40</sup> KOTVAL, J. P. and GRAY, L. H., *ibid.*, 1947, **48**, 135
- <sup>41</sup> GILES, N. H. and BEATTY, A. V., *Science*, 1950, **112**, 643
- <sup>42</sup> THODAY, J. M. and READ, J., *Nature*, 1947, **160**, 608
- <sup>43</sup> BAKER, K. W. and von HALLE, E. S., *Proc. nat. Acad. Sci., Wash.*, 1953, **39**, 152
- <sup>44</sup> PUGSLEY, A. T., ODDIE, T. H. and EDDY, C. E., *Proc. roy. Soc.*, 1933, **118B**, 276
- <sup>45</sup> SLAUGHTER, J. C. and FAILLA, G., *Radiology*, 1942, **39**, 663
- <sup>46</sup> BARRON, E. G., FLOOD, V. and GASVODA, B., *Biol. Bull., Wood's Hole*, 1949, **97**, 51
- <sup>47</sup> GRAY, L. H., *Progr. Biophys.*, 1952, **2**, 240
- <sup>48</sup> ZIRKLE, R. E., *Symposium on Radiobiology*, p. 333, Wiley, New York, 1952
- <sup>49</sup> GRAY, L. H., *Brit. J. Radiol.*, 1953, **26**, 609
- <sup>50</sup> HOPPENBROEK, A., *Symposium on Radiobiology*, p. 285, Wiley, New York, 1952
- <sup>51</sup> GELIN, O. E., *Hereditas, Lund*, 1941, **27**, 209

DIRECT AND INDIRECT ACTION

- 52 PETRY, E., *Biochem. Z.*, 1922, **128**, 326
- 53 HENSHAW, P. S. and FRANCIS, D. S., *J. cell. comp. Physiol.*, 1935, **7**, 173
- 54 WERTZ, E., *Strahlentherapie*, 1940, **67**, 700
- 55 PATT, H. M., *Physiol. Rev.*, 1953, **33**, 35
- 56 LAMBERT, J., *Arch. Biol. Paris*, 1933, **44**, 621
- 57 FIRKET, J. and COMHAIRE, S., *Bull. Acad. Méd. Belg.*, 1929, p. 93
- 58 LECLOUX, J., VIVARIO, R. and FIRKET, J., *C.R. Soc. Biol. Paris*, 1927, **97**, 1823
- 59 STAPLETON, G. E., BILLEN, D. and HOLLAENDER, A., *J. cell. comp. Physiol.*, 1953, **41**, 345
- 60 KIRBY-SMITH, J. S. and DANIELS, D. S., *Genetics*, 1952, **37**, 596
- 61 GRAY, L. H., CONGER, A. D., EBERT M., HORNSEY, S. and SCOTT, O. C. A., *Brit. J. Radiol.*, 1953, **26**, 638
- 62 THODAY, J. M., *J. Genetics*, 1942, **43**, 189
- 63 GRAY, L. H., READ, J. and POYNTER, M., *Brit. J. Radiol.*, 1943, **16**, 125
- 64 EHRENBERG, L., GUSTAFSSON, A. and NYBOM, N., *Acta chem. scand.*, 1952, **6**, 1554
- 65 EHRENBERG, L., GUSTAFSSON, A. and NYBOM, N., *Ark. Bot.*, 2, 1952, **1**, 557
- 66 STAPLETON, G. E., HOLLAENDER, A. and MARTIN, F. L., *J. cell comp. Physiol.*, 1952, **39** (Suppl. 1), 87
- 67 EVANS, T. C., *Nucleonics*, 1949, **4** (No. 3), 2  
BRENNAN, J. T., HARRIS, P. S., CARTER, R. E. and LANGHAM, W. H.,  
*Los Alamos Lab. Rep.* 1952, 1408
- MITCHELL, J. S., *Brit. J. Radiol.*, 1947, **20**, 368
- TOBIAS, C. A., *A.E.C.D. 2099-A (U.S. Rep.)*
- 68 TIMOFEEFF-RESSOVSKY, N. W. and ZIMMER, K. G., *Naturwissenschaften*, 1938, **26**, 108  
GILES, N. H., *Genetics*, 1943, **28**, 398
- DEMPSTER, E. R., *Proc. nat. Acad. Sci., Wash.*, 1941, **27**, 249
- 69 FANO, U., *Genetics*, 1944, **29**, 361
- 70 BAKER, K. W. and von HALLE, E., *Science*, 1954, **119**, 46
- 71 NEARY, J. N., MUNSON, R. J. and MOLE, R. H., *Nature*, 1954, **171**, 256  
EVAN, T. C. and LEINFELDER, P. J., *Radiation Res.*, 1954, **1**, 130
- 72 GRAY, L. H., MOTTRAM, J. C. and READ, J., *Brit. J. Radiol.*, 1948, **21**, 5
- 73 MACKEY, H., *Hereditas, Lund*, 1951, **37**, 421; *Arch. J. Bot.*, Ser. 2, 1952,  
**1**, 545
- 74 GRAY, L. H. and SCHOLES, R., *Brit. J. Radiol.*, 1951, **24**, 82
- 75 ZIRKLE, R. E., AEBERSOLD, P. C. and DEMPSTER, E. R., *Amer. J. Cancer*, 1937, **29**, 556
- 76 TAHMISIAN, T. N. and VOGEL, H. H., *Proc. Soc. exp. Biol. Med.*, 1953, **84**, 538
- 77 ZIRKLE, K. E. and TOBIAS, C. A., *Arch. Biochem.*, 1953, **47**, 282
- 78 ALEXANDER, P. and CHARLESBY, A., *Radiobiol. Symp. (Liège) publ.*  
Butterworth 1955

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

### INTRODUCTION

THE effect of ionizing radiations on water and aqueous systems is of immediate importance to radiobiology as a complete understanding of indirect action can only be obtained when the radiation chemistry of water has been elucidated. In spite of careful experimental work which has been done in this field, almost since the discovery of radium, no wholly satisfactory picture can yet be put forward for the changes which occur when pure water\* is irradiated and a complete treatment of the reactions occurring in aqueous systems is not possible. A number of basic reactions has been established on a qualitative basis and has proved most useful for the interpretation of radiobiological processes. In this chapter, after a brief historical survey, the type of free radicals formed by ionizing radiations in water and the influence of the specific ionization on the number of radicals which are available for reaction with a solute will be discussed. The general nature of the reaction of these free radicals with different dissolved substances will be illustrated by examples which are of possible biological significance. The reaction of macromolecules will be dealt with in detail in the following chapter. Finally, some unusual physicochemical changes produced in colloids will be described.

### HISTORICAL DEVELOPMENT

As early as 1901 CURIE and DEBIERNE<sup>1</sup> observed a continuous evolution of oxygen and hydrogen from solutions of radium salts and this reaction was studied in more detail by RAMSAY and SODDY<sup>2</sup>. An analysis of the gases revealed that the decomposition was not stoichiometric and that there was an excess of hydrogen<sup>3</sup>. This

---

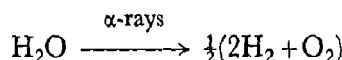
\* The term pure water implies that the water has been carefully purified by repeated distillations and completely degassed.

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

anomaly was resolved by KIRNBAUM<sup>4</sup>, who found that the solution of radium salts contained an amount of hydrogen peroxide equivalent to the deficiency of oxygen.

The reverse reaction, the formation of water from hydrogen and oxygen by admixture of radon, was studied in considerable detail by CAMERON and RAMSAY<sup>5</sup>, who found that the following rules were obeyed: (i) 'that every molecule of emanation (radon) disintegrating produces a definite chemical effect', and (ii) 'that each molecule of emanation (radon) as it disintegrates, produces the same amount of chemical change'. At the time of this investigation it was generally believed that the action of radon was that of catalyst, and it had not been realized that the energy for the reactions was provided by the concomitant radiations. These experimentally derived relationships were therefore unexpected, although it is now known that, subject to some qualifications, they are quite general and fundamental for radiation chemistry.

From the data of Ramsay, BRAGG<sup>6</sup> calculated that the number of water molecules decomposed was almost identical with the number of ions which a similar amount of radon would produce in air. This agreement was referred to by Bragg as a 'curious parallelism in numbers' which indicates that an  $\alpha$ -particle may in the course of removing of one or more electrons cause 'a more complete disruption of the molecule or even the atom'. This appears to be the first suggestion that the chemical changes produced by radon were the result of ionization by  $\alpha$ -particles. LIND<sup>7</sup> recalculated the available data in terms of ionic yield (see p. 36) and for the reaction



the M/N values were 0.8 to 1 for liquid water; 0.05 to 0.1 for ice; and less than 0.01 for water vapour. For the reverse reaction (*i.e.* formation of water) the high value of M/N = 4 was found, and this may explain the low yield for the decomposition of water vapour. The presence of oxygen does not influence the formation of hydrogen peroxide by  $\alpha$ -rays in contrast to the results obtained with x-rays (see p. 107). The general principle, on which current theories are based, that the action of ionizing radiation on water is the establishment of an equilibrium first became apparent in experiments from Mme Curie's laboratory, where DUANE and SCHEUER<sup>8</sup> studied the decomposition of water by  $\alpha$ -rays in great detail.

Concurrently with these investigations the chemical effects of x-rays on different solutes was studied, notably by KAILAN<sup>9</sup>. In

#### HISTORICAL DEVELOPMENT

aqueous solutions hydrogen peroxide was decomposed, the iodide anion oxidized to iodine and most significantly benzoic acid was obtained by irradiating toluene dissolved in water<sup>10</sup>. Lind realized that these reactions in aqueous solution were 'indirect' and wrote concerning the oxidation of iodide: 'The radiation first acts on water to produce an activated (nascent) form of oxygen, which reacts with potassium iodide in a secondary reaction'. This concept was firmly established independently by FRICKE<sup>11</sup> and RISSE<sup>12</sup> in a series of investigations on the effect of x-rays on aqueous systems. Their experiments were notable for the high experimental skill and the extreme care used to avoid contamination by impurities.

Unlike  $\alpha$ -particles the less densely ionizing radiations do not decompose pure degassed water, and both Fricke and Risse were unable to detect the formation of hydrogen peroxide or hydrogen gas after large doses of x-rays\*. Yet chemical reactions were induced by x-rays under anaerobic conditions; inorganic as well as organic solutes were decomposed, oxidized or reduced often with a yield of M/N = 1. Fricke clearly established that these reactions were indirect (*i.e.* yield independent of solute concentration) and considered that the reactive species was 'activated' water. The problem of defining 'activated' water in chemical terms has not been wholly solved, although the suggestion made by Risse<sup>13</sup> in 1929 that x-rays decompose water into OH and H radicals has, after lying fallow for some years, found wide acceptance. Risse pointed out that the failure of x-rays to decompose pure water could be interpreted in terms of recombination of H and OH radicals and that the increased yield in the presence of oxygen would be due to the formation of a peroxide radical (*i.e.* HO<sub>2</sub><sup>·</sup>). The full usefulness of the free radical hypothesis was appreciated only after the reactivity of these entities in water was better understood when the suggestion that x-rays decompose water into H<sup>·</sup> and OH<sup>·</sup> was put forward anew in 1944 by WEISS<sup>14</sup>. Direct evidence for the existence of the OH radical in radiochemical reactions was provided by DAINTON<sup>15</sup> in 1948, but the formation of a hydrogen atom has not been proved<sup>96</sup>. The mechanism of the formation of these radicals, their distribution in space, and the number formed by a given dose, are all fundamental problems which have not yet been satisfactorily solved.

---

\* Hydrogen peroxide is produced on irradiating water with x-rays in the presence of oxygen (see p. 107) or in deaerated solutions in the presence of substances (*cf.* bromide) which can undergo oxidations (see p. 109).

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

### THE FORMATION OF FREE RADICALS

The ions formed by electron impact on water vapour have been quantitatively determined in the mass-spectrometer<sup>16</sup>. In order of abundance the products are  $\text{HO}_2^+$ ,  $\text{HO}^+$ ,  $\text{H}^+$  and  $\text{H}_3\text{O}^+$ ; very small amounts of  $\text{O}^+$  and  $\text{H}_2^+$  have also been detected.  $\text{H}_2\text{O}^+$  requires the least energy ( $\sim 13$  eV) for its formation and only about 40 per cent of the energy needed to form an ion pair (32.5 or 35 eV) is used in this process. Only a small fraction of this excess energy can be retained by ejected electrons—since these would otherwise produce further ionization—and most of it will be used to produce electronically excited water molecules. Charge neutralization of the ions formed will also lead to excited molecules of high energy content which can dissociate into H and OH radicals.

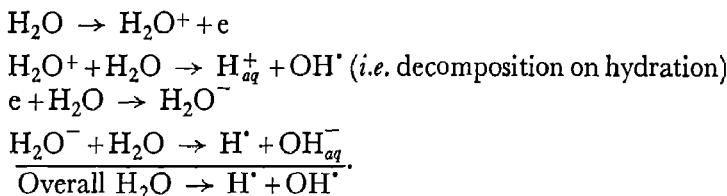
DAINTON<sup>15</sup> has analysed the data available for water vapour to see how far it can be used to determine the processes likely to occur in liquid water and aqueous solutions. An immediate difficulty when dealing with reactions in water is that the number of ion pairs formed in liquids cannot be determined. All calculations are based on the hypothesis that the energy required to form an ion pair is the same in condensed systems as in gases (see p. 23). On this basis exposure of water to a dose of 1 r of x-rays will produce a concentration of  $3 \times 10^{-9}$  M of ion pairs.  $\text{H}_2\text{O}^+$  is believed to be the predominant species formed in water and is thought to dissociate to give an OH radical. DAINTON and COLLINSON<sup>17</sup> suggest that  $\text{OH}^+$  radical ions are also produced in the condensed phase and that they are responsible for the high 'redox potential' observed in irradiated water (see p. 101). The ions immediately formed become hydrated and the energy made available in this process is a determining factor in the subsequent fate of the ion.

The point about which there is least information is the behaviour of the 'thermal' electron produced with the  $\text{H}_2\text{O}^+$  ion. This can either be captured by another water molecule to give a negative ion, or recombine with the  $\text{H}_2\text{O}^+$  to give an excited molecule. The mechanism of radical formation is critically dependent on the model chosen. The possibility that the electron may have an independent existence in water long enough to enter into chemical reaction has not been quantitatively examined, but cannot be excluded (see p. 86).

The first mechanism for the formation of the radicals from the ions was proposed by LEA<sup>18</sup> in 1947. A pair of positive and negative water ions was postulated as the direct product of the ionization. These decomposed on becoming hydrated within the relaxation

### THE FORMATION OF FREE RADICALS

time of water (*i.e.*  $10^{-11}$  sec) to give the radicals first postulated by RISSE<sup>13</sup>. Within this period no significant diffusion of the ions can occur so that the radicals are formed at the same sites as the ions. The complete reaction is represented as follows:



The most notable feature of this mechanism is that the two radicals are not formed close together as, according to LEA<sup>18</sup>, the slow electron has, on a molecular scale, an appreciable free path before it is captured to give a negative ion\*. According to DALE, GRAY and MEREDITH<sup>19</sup> the separation is of the order of 15 m $\mu$  in water (*i.e.* the radicals will be separated by about 70 water molecules).

### EXCITED MOLECULES

The behaviour of the excited water molecules is not known. One of the possible reactions which they could undergo is dissociation into H and OH radicals. The energy required to do this, approx. 5 eV, is probably available (*i.e.*  $W = 32.5$  eV; energy required to ionize water from ionization potential measurement is 16 eV; thus if for each ion pair formed three excited molecules are produced, each one would have an energy of somewhat more than 5 eV.) It is unlikely that the conversion of the energy deposited in an electronically disturbed atom would be transferred with high efficiency into the H—O bond, and general consideration suggests that deactivation processes in liquids reduce the number of molecules having sufficient energy to dissociate. Also the 'cage effect' (see p. 40) would bring about the immediate recombination of H and OH radicals produced by dissociation of a water molecule having an energy content sufficient, but not greatly in excess of, that required for dissociation. LEA<sup>21</sup> and others were of the opinion that few H and OH radicals, diffusing separately through the medium, are contributed by excited molecules and that the number of chemical reactions occurring in water was determined by the number of ionizations. This hypothesis received powerful quantitative

\* This view has recently been challenged (see p. 85) but GRAY<sup>20</sup> in a recent publication adheres to the value for the separation for the positive and negative ion which have been deduced from cloud chamber studies (see p. 22).

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

support from investigations in aqueous systems, notably the experiments of FRICKE and HART<sup>22, 23</sup> which provide many examples of reactions for which the ionic yield M/N is close to 1 (*i.e.* 'G' value for radical formation is 3).

Recently significantly higher 'G' values have been noted for the reduction of ceric to cerous ions (HAISSINSKY<sup>55</sup>), and the decomposition of hydrogen peroxide<sup>24</sup> with x-rays and  $\gamma$ -rays.<sup>1</sup> There appear to be two possibilities to resolve this discrepancy; (i) the number of each primary radical produced per 100 eV of energy absorbed is greater than three. This position could arise in two ways: (*a*) if dissociation of excited water molecules contributes radicals capable of reacting with the solute, or (*b*) if the energy required to form an ion pair is less than 32.5 eV\*. According to this view, which is held by DAITON and ROWBOTTOM<sup>25</sup>, the ionic yield of 1 found for many reactions is fortuitous, and is due to incomplete utilization of the radicals or the occurrence of an unknown back reaction. (ii) Two types of 'activated water' are formed; the more reactive (probably H and OH radicals) is produced by ionization, and the other from excited molecules. The high 'G' values observed with some processes, such as the decomposition of hydrogen peroxide, are due to the fact that both kinds of 'activated water' can take part, while others, notably the decomposition of organic compounds, can only be brought about by the more reactive form†.

If the second mechanism is accepted then the reaction scheme outlined on p. 83 for the formation of free radicals could be retained. Other experimental evidence, and in particular the formation of molecular hydrogen and hydrogen peroxide, which occurs as a by-product of many radiochemical reactions induced by radiations of low specific ionization, suggests that this mechanism is inadequate. These difficulties may resolve themselves when more is known about the fate of the excited molecules and a reaction scheme based on the dissociation of distinct positive and negative water ions may be correct. On the other hand, an entirely new approach, as has been proposed by BURTON, MAGEE and SAMUEL (B.M.S.)<sup>28</sup>, may be

\* It is possible that these two mechanisms are not distinct, as there are quantum mechanical grounds for believing that in a liquid the energy levels of excited and ionized molecules overlap so that a separation of energy absorption into two processes may be misleading<sup>26</sup>.

† The energy required to dissociate  $H_2O_2 \rightarrow 2OH^-$  is 56 kcal/mole, while 118 kcal/mole are required to dissociate water into  $H^+$  and  $OH^-$ . Consequently an excited water molecule, which has insufficient energy to dissociate into free radicals could still decompose hydrogen peroxide. No experimental evidence is available for the existence of an excited water molecule of sufficiently long life to take part in reactions with solutes (*i.e.*  $10^{-5}$  to  $10^{-4}$  sec) but NIRA<sup>27</sup> has postulated an entity on theoretical grounds which fulfils these requirements.

### EXCITED MOLECULES

needed. According to the B.M.S. hypothesis the free radicals, H and OH, are formed solely by dissociation of excited water molecules. Two types of excited species are formed  $M^*$  and  $M^\dagger$ .  $M^*$  is produced in primary processes by excitation and is relatively stable. It does not dissociate into free radicals, either because it loses its energy before dissociation, or because immediate recombination occurs, as there is insufficient energy for the radicals to be able to escape from the 'cage'. The lifetime of  $M^*$  is sufficiently long (at least  $10^{-5}$  sec) for it to be able to take part in chemical reaction, though its reactivity is limited.  $M^\dagger$  is produced by charge neutralization and has a large energy content, so that the radicals formed by dissociation have sufficient kinetic energy to escape from the cage effect. The distance between successive  $M^\dagger$  molecules varies with the specific ionization of the radiation and some representative values for water are given in *Table I*. The same values will apply to the separation of OH ions along the track according to Lea's hypothesis of immediate dissociation of the  $H_2O^+$  ion.

### THE FATE OF THE 'THERMAL' ELECTRON

The essential difference between the B.M.S. and the Lea mechanism for the formation of free radicals in water is the behaviour of the thermal electron ejected from a water molecule and having an energy of a few eV or less. According to B.M.S., the  $H_2O^+$  ion will recapture the ejected electron before this has had time to diffuse out of range. LEA<sup>18, 21</sup> and GRAY<sup>19</sup> have carried out rather approximate calculations based on the electron cross-section data of JAFFE<sup>29</sup> and Wilson cloud chamber pictures, which leads them to the view that the kinetic energy of the ejected electron is sufficient for it to escape from the influence of the positive ion (*i.e.* to travel beyond a critical distance where the electrical-coulombic-attraction is smaller than the thermal energy). As already mentioned Gray believes that in water the electron travels an average distance of 150 Å before it is captured to form the negative ion.

More recently SAMUEL and MAGEE<sup>30</sup> using a simple but convincing model have calculated that the electron loses its energy very rapidly to surrounding water molecules and that it cannot in fact escape from the strong field of the ion. After travelling a distance less than 20 Å the electron has lost sufficient energy to come into the attraction field, and approximately  $10^{-13}$  sec is taken up for the charge neutralization process (*i.e.* for the electron to lose its kinetic energy and to return to the vicinity of the positive ion). This time interval is too short for the positive ion to dissociate.

### THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

Therefore, if this theory is correct, all radiation chemical reactions in solids and liquids proceed via excited molecules and their free radical dissociation products; ions play no part, as their lifetime is too short for them to enter into chemical reactions.

The premise on which the calculations of Samuel and Magee are based has not been accepted by PLATZMANN and FROELICH<sup>31</sup>, who believe that charge neutralization is a very infrequent process. These authors have made a detailed study of the motion of slow electrons in dipolar systems, such as water, and conclude that the rate of energy loss is even less than that used by Lea and Gray in their calculations. Accordingly, even the formation of the  $\text{H}_2\text{O}^-$  ion by an electron capture process will be relatively slow, and it is not impossible that the electron may have an independent existence of the order of  $10^{-5}$  sec. This time interval is long enough for the electron to react with dissolved substances.

An alternative method of obtaining a relatively persistent atmosphere of electrons is to assume<sup>24</sup> that the electron is captured by water, but that the resultant  $\text{H}_2\text{O}^-$  ion does not have time to decompose before the electron is passed on to another water molecule. This postulate is not improbable since the outer electronic orbits of water overlap in the liquid phase. Probably a time interval of  $10^{-15}$  to  $10^{-14}$  sec would be necessary for transfer from one molecule to another while the  $\text{H}_2\text{O}^-$  ion requires  $10^{-11}$  sec (time for relaxation of dipoles) before it can dissociate. This model is equivalent to the formation of an electron band of the type which exists in metals and semi-conductors. In the presence of a molecule of high electron affinity the electron would be trapped and no longer passed from water molecule to water molecule, as the symmetry of the system would be lost.

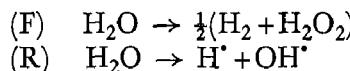
At present there seems to be insufficient data to decide between the possible processes. At first sight the difference between the Lea and B.M.S. theory may appear to be only formal as both provide for the formation of an H and OH radical per ionization. This is not so; the two mechanisms lead to different distribution in space for the H and OH radicals, which should significantly influence the chemical effects produced by radiation having a high specific ionization.

### QUANTITATIVE ASPECTS

Extensive studies of the radiation chemistry of water have led ALLEN<sup>32</sup> to suggest that the primary effect of ionizing radiations should be regarded as two processes, the forward reaction (F) and

### QUANTITATIVE ASPECTS

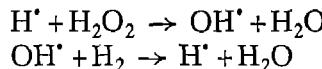
the radical reaction (R). In early experiments FRICKE<sup>33</sup> found that the oxidation of simple anions such as bromide or iodide by x-rays in pure water was accompanied by the formation of *equal* amounts of hydrogen and hydrogen peroxide. This observation provided the first indication for a 'molecular' reaction. Without implying any mechanism Allen writes these processes as follows:



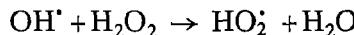
The respective yields per 100 eV are referred to as  $G_F$  and  $G_R$ .

Allen does not claim that these reactions occur by different mechanisms and it is not suggested that one water molecule of water decomposes by the (F) and another by the (R) process. The most plausible interpretation is that a fraction of the free radicals formed (by any of the mechanisms discussed in the previous section) interact immediately with one another to give molecular products and are never available for reaction with solute\*. The remainder of the free radicals diffuse into the solution where they can interact with other materials if these are present. In pure water the 'escaped' radicals recombine or react with the molecular products.

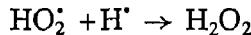
According to Allen the failure to detect hydrogen peroxide and hydrogen when *pure* water is irradiated with x-rays (see p. 81) is due to the destruction of the molecular products by the free radicals from the (R) reaction. ALLEN and his collaborators<sup>26</sup> have found that initially some hydrogen is produced by  $\gamma$ - and x-rays, but the rate of appearance quickly decreases and an equilibrium concentration, which is at the limit of detection, is soon attained. The molecular products are probably destroyed by this chain reaction



which is terminated by recombination of  $\text{H}^\bullet$  and  $\text{OH}^\bullet$ . There is some evidence<sup>26</sup> that the chain may also be broken by the well-established reaction



The  $\text{HO}_2^\bullet$  radical is reduced back to hydrogen peroxide



which then rejoins the chain process.

---

\* A reaction between two excited water molecules could also lead to the formation of  $\text{H}_2$  and  $\text{H}_2\text{O}_2$ . A bimolecular process can, however, be excluded on kinetic grounds for all except densely ionizing radiations since the probability of two excited molecules colliding is insignificant at the low local concentrations in x-ray and  $\gamma$ -ray tracks.

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

The paradox that no hydrogen peroxide (or hydrogen gas) is formed in pure deaerated water but that these molecular products are formed when a reducing substrate is oxidized can be understood in terms of an (F) and (R) reaction. Decomposition of the hydrogen

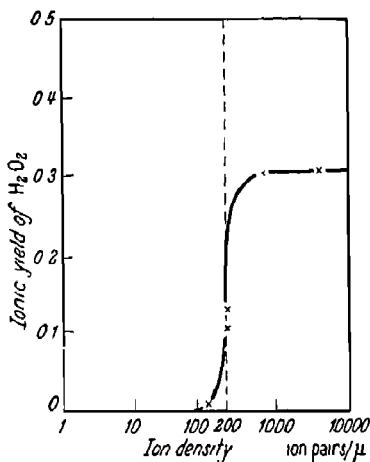


Figure 1. Influence of the specific ionization of the radiation on the amount of  $H_2O_2$  produced (expressed as ionic yield) produced by irradiating pure oxygen-free water.<sup>34</sup>

peroxide competes with oxidation of the substrate for the available OH radicals.

With  $\alpha$ -particles the position is different and both hydrogen and hydrogen peroxide are continually produced on the irradiation in pure water and no equilibrium is reached. Presumably the back

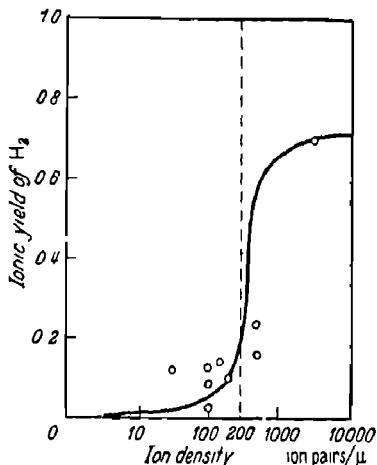


Figure 2. Influence of the specific ionization of the radiation on the amount of hydrogen gas (expressed as ionic yield) produced by irradiating pure oxygen-free water.<sup>34</sup>

reaction cannot cope with the proportionally much greater forward reaction. BONET-MAURY<sup>34</sup> has correlated the available data for the formation of molecular products in 'pure' water by radiations of different specific ionization as shown in Figures 1 and 2. A sharp change occurs at ionization density of approximately 200 ion/ $\mu$ .

### QUANTITATIVE ASPECTS

Radiations below this value behave like x-rays while those above this value behave like  $\alpha$ -rays.

*Relative amounts of (F) and (R) reactions*—For chemical reactions the division into the (F) and (R) processes is justified, even if the fundamental mechanism is the same, as the radicals giving rise to the molecular products (F) can never take part in any other reaction. (F) depends upon the ionization density of the radiations used and is largest for those of highest specific ionization.

From the total yield of the reaction the 'G' value (*i.e.*  $G_F + G_R$ ) is obtained. If the substrate reacts completely with all the radicals available for the (R) reaction then  $G_F$  can be experimentally determined by measuring the amount of hydrogen and hydrogen peroxide produced. In general the hydrogen evolution can be measured more easily and in a number of different systems this gave  $G_F$  of the order of 0.8 for radiations of low specific ionization. For the oxidation of ferrous sulphate in aerated solutions 'G' and  $G_F$  values have been obtained for radiations of different specific ionizations (see *Table II*). The change of  $G_F/G_F + G_R$  with specific ionizations has been calculated by SAMUEL and MAGEE<sup>30</sup> and there is close agreement between the theoretical and experimental values (see *Table I*). The calculation of these  $G_F$  values from the experi-

*Table I. Oxidation of Air saturated Solutions of Ferrous Sulphate (in 0.8N H<sub>2</sub>SO<sub>4</sub>) by different ionizing Radiations*

<i>Radiation source</i>	<i>Average ionization density ionizations per <math>\mu</math> length of track</i>	$G_{Fe^{+++}}$	$\frac{G_F}{G_F + G_R}$ <i>exptl.</i>	<i>Calc.</i>
Po $\alpha$ . .	4000	5.9	0.81	0.89
Tritium electron . .	200	12.9	0.30	0.31
$\gamma$ -rays . .	100	20	0.23*	0.23

\* Average of value of HART<sup>37</sup> 0.21 and HOCHANADEL<sup>38</sup> 0.25.

*Table II. The number of water molecules decomposed by 100 eV of x- or  $\gamma$ - rays (*i.e.*  $G_{H_2O} = G_F + G_R$ ) as determined by studying different reactions*

<i>System studied</i>	$G_{H_2O}$
<i>Decompr. of organic materials; calculated by method of ALDER and EYRING<sup>40</sup></i> . .	3.6
<i>Reduction of O<sub>2</sub> to H<sub>2</sub>O<sub>2</sub><sup>38</sup></i> . .	3.7
<i>Decomposition of formic acid<sup>37</sup></i> . .	3.8
<i>Oxidation of benzene<sup>41</sup></i> . .	2.9
<i>Decomposition of H<sub>2</sub>O<sub>2</sub><sup>24</sup></i> . .	13.4
<i>Reduction of ceric sulphate<sup>55</sup></i> . .	12.9

mental results is based on two assumptions; firstly that the molecular mechanism shown on p. 87 is correct; secondly that all the radicals which are in a position to do so react the substrate and that none combine with one another. Both these postulates are supported by the available data, but decisive proof is lacking.

DAINTON and SUTTON<sup>36</sup> have determined the production of hydrogen peroxide in the ferrous sulphate oxidation with x-rays by following the rate of oxidation after the irradiation is complete (see Figure 3). Ferrous iron is oxidized by hydrogen peroxide, and at low concentrations this reaction is relatively slow. From the kinetics of the after-effect the quantity of hydrogen peroxide formed could be calculated, giving  $G_F = 1.2$ .\*

If the  $G_F$  value is known the  $G_R$  value is obtained by difference from the 'G' value. For hard radiations the oxidation of ferrous sulphate has a value of  $G_R = 5.5$  while lower values of 3.5 to 3.6 have

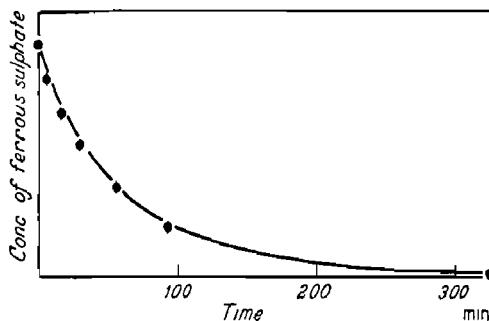


Figure 3. Post-irradiation oxidation of ferrous sulphate due to hydrogen peroxide produced by the 'molecular process'<sup>36</sup>

been found for the oxidation of formic acid<sup>37</sup> and the production of  $H_2O_2$  in aerated water<sup>38</sup>. For the decomposition of hydrogen peroxide, on the other hand, DAINTON and ROWBOTTOM<sup>25</sup> obtain the exceptionally high value of  $G_R = 12$ . Experiments on the decomposition of peroxide are extremely difficult and different workers have obtained results which are completely at variance<sup>39</sup>. Until this lack of agreement has been resolved it is unwise to place too

\* The value of  $G_F = 0.8$  obtained from the formation by x-rays and  $\gamma$ -rays of molecular hydrogen is based on the results of investigators whose experimental work is recognized to be of the highest precision and who have experience in dealing with the difficult problems of high purity, which is essential if quantitative data are to be obtained. The value for  $G_F$  from  $H_2O_2$  formation is considerably higher than that found for molecular hydrogen formation and may mean that the two molecular products are not formed in equal amounts. The data available is too limited for this point to be decided. At present the molecular reaction can still be represented as  $H_2O \rightarrow \frac{1}{2}(H_2 + H_2O_2)$ .

### QUANTITATIVE ASPECTS

much reliance on this high  $G_R$  value. However, even if the experimental results are confirmed, it is possible that part of the decomposition is due to excited water molecules, which are not, however, energetic enough to decompose formic acid or react with oxygen to give hydrogen peroxide (see footnote p. 84). The intermediate value found for ferrous sulphate may be attributed to the fact that excited water molecules can take part in this reaction, but are less effective than in the decomposition of peroxide.

Although  $G_R$  and  $G_F$  values are only known for a few reactions, ALDER and EYRING<sup>40</sup> have developed a kinetic treatment which enables them to determine the number of water molecules dissociated into radicals from the relationship between chemical yield and solute concentration. They have analysed the data for the x-ray induced decomposition of aqueous solutions of glutathione, fluorescein, carboxypeptidase, ascorbic acid and riboflavin. Weighting equally the rather scattered values they obtain an average of 3.6 water molecules decomposed per 100 eV. Consideration of all the data shows that if the high values of  $G_R$  found for peroxide and ferrous sulphate are attributed to excited molecules, the energy required to decompose one water molecule (*i.e.*  $100 \text{ eV}/G_F + G_R$ ) into radicals is of the order of 30 eV.

The surprising conclusion (*cf. Table II*) is that the simple ionic yield treatment in which it was assumed that one ion pair was formed per 32.5 eV and that this in turn gave one pair of radicals is in agreement with the new data even when treated by much more general methods. If this interpretation is accepted excited water molecules probably do not play an important role in indirect biological effects as they can only take part in relatively facile reactions and cannot intervene in processes, such as the decomposition of organic substances, which are more likely to be of biological importance.

### SPATIAL DISTRIBUTION OF THE IONS FORMED BY DIFFERENT RADIATIONS

A knowledge of the spatial distribution of the ions is necessary for an understanding of the kinetics of radiochemical reactions and of the quantitative\* differences found between radiations of different

\* Although claims have been made that densely ionizing radiations produce radiochemical reactions which are qualitatively different, none of these have been substantiated in subsequent more detailed studies. For example, STEIN and WEISS<sup>41</sup> claimed that irradiation of benzene in water by neutrons and  $\alpha$ -particles, but not by x-rays, resulted in the formation of a dialdehyde. Subsequent studies<sup>42</sup> showed that these authors had failed to appreciate the full complexity of the reaction of this system with x-rays and had not noticed a substantial after-effect. This was relatively more important in the neutron and  $\alpha$ -ray irradiations as these were of longer duration than the experiments with x-rays.

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

specific ionizations. In aqueous solution reaction of the substrate with the radicals is always in competition with radical recombination. The solute molecules can only take part in the reaction if its concentration is of the same order as that of the free radicals *at the site of formation*.

It has been seen in the previous section that the available data can be explained by postulating that a fraction of the radicals always recombine to give molecular products whatever the concentration of the solute. The remaining radicals diffuse freely and can either react with the solute or recombine. FRICKE and HART<sup>22, 23</sup> showed that for all the reactions (oxidation, reductions, dissociations) produced by x-rays in aqueous solutions, the yield was constant once the concentration exceeded a minimum value which lay between  $10^{-3}$  to  $10^{-2}$  M (see Figure 4). More recent work has amply con-

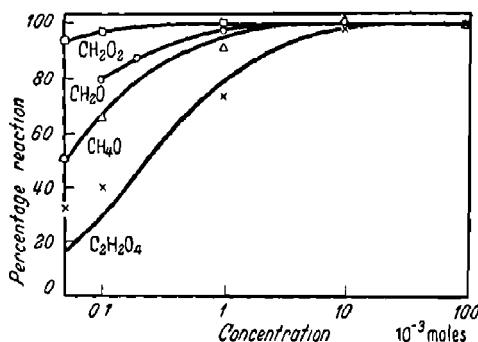


Figure 4. Influence of concentration of radiochemical yield for the decomposition of formic acid, formaldehyde, methyl alcohol and oxalic acid<sup>23</sup>

firmed these results and the only well-established exceptions are the deamination of amino-acids and the decomposition of hydrogen peroxide for which the yield increases with concentration up to the highest obtainable (see p. 113); these anomalies are almost certainly due to the occurrence of chain reactions.

The simplest interpretation for the constant yield is that beyond a minimum concentration every available radical reacts with the solute and none are lost by recombination\*. A quantitative treatment of these problems is complex since the distribution of the ions is far from uniform. Along an approximately linear path of ionizing particles, ionizations occur at intervals which are determined by the

\* This does not mean that the number of molecules changed corresponds to the number of radicals of one type formed as there is always another radical which may drive the reaction in the opposite direction. The low value of ' $G$ ' = 1.2 for the oxidation of nitrite, arsenite, ferrocyanide and selenite by x-rays in deaerated water<sup>33</sup> can probably be explained in this way.

## Spatial Distribution of the Ions Formed by Different Radiations

charge and velocity of the particle (see p. 5). Secondary ionizations occur within a few Ångstrom units of the primary ionization (clusters or spurs) and occasionally at greater distances ( $\delta$ -rays). With densely ionizing radiations (e.g.  $\alpha$ -rays) the clusters are formed so closely together that they cannot be distinguished individually and the ions can be considered as uniformly localized in a cylindrical track;  $\delta$ -rays, however, still have to be considered separately as they fork out from the main track (see *Figure 9*, p. 24). The distribution of the original ionizations can be calculated quantitatively and there are Wilson cloud chamber photographs to substantiate the results.

Before this data can be used to calculate the distribution of the H and OH radicals in water a decision has to be made by what mechanism these are formed. The theory of Lea assumes that separate positive and negative ions are formed in the liquid in the same way as in gases (see p. 24) and that the radicals are produced at the site of the formation of the ions. According to this view OH radicals and  $H^+$  ions are formed along the main tracks and the  $\delta$ -tracks by the process  $H_2O^+ \rightarrow H^+ + OH^-$ . The H atoms and  $OH^-$  ions ( $H_2O^- \rightarrow H + OH^-$ ) are produced away from the track since the ejected electron travels a definite distance before attaching itself to a molecule. A value for these distances was obtained by extrapolation from Wilson cloud chamber pictures (see p. 22). According to the alternative theory of Burton *et al.* (see p. 85) which postulates that the H and OH radicals are produced from the same water molecule, the radicals will be present as a random mixture within the tracks.

Independently LEA<sup>18</sup>, and DALE, GRAY and MEREDITH<sup>19</sup> calculated the spatial distribution of the radicals based on the assumption that the two radicals are produced from different water molecules. For this computation values for the range of the ejected electron in water have to be assumed since no direct measurements can be made. Consideration of the data available from experiments in gases suggested that a value of 150 Å is reasonable. With sparsely ionizing radiation (e.g. 60 kV x-rays and harder) the separation along the clusters is sufficiently great for the distribution of the H and OH radicals to be uniform so that there is no qualitative difference between the two theories of radical formation (see *Figure 5*). With more densely ionizing radiation the Lea hypothesis leads to the concept of a central core\* containing a high concentra-

\* The energy released in this central core will be sufficient to melt ice. On the passage of an  $\alpha$ -particle through a frozen structure it is possible, therefore, that some diffusion of radicals may occur. This point has to be borne in mind when the freezing test is used to establish direct action.

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

tion of OH radicals surrounded by a relatively diffuse column of H atoms at a much lower concentration; the values are given in *Table III*. Associated with the OH radicals are hydrogen ions so that the central core will be a region of high acidity and James Franck has suggested that this may contribute to the biological effect of ionizing radiations. In the much more diffuse cylinder of H atoms, the pH will be increased by the associated OH ions but this

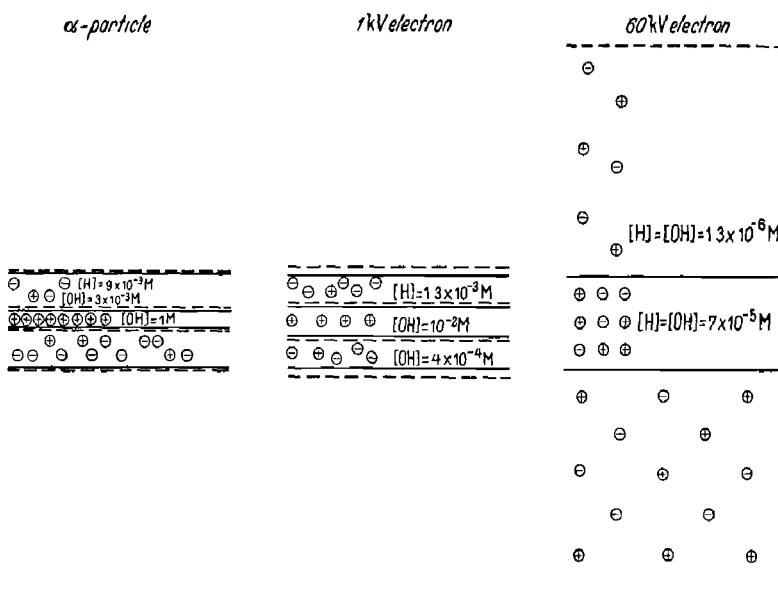


Figure 5. Diagrammatic representation, according to GRAY<sup>20</sup> of tracks of ionizing particles in water assuming the ejected electrons are captured by a water molecule to form a negative ion 15 m $\mu$  distant from the parent positive ion. The inner solid line represents the initial distribution of OH radicals and the outer solid line the initial distribution of H radicals. The dotted lines show how far the radicals will spread in the time necessary for 50 per cent of the radicals to collide with one another (i.e. for 50 per cent of the radicals to disappear by recombining to give molecular products). For  $\alpha$ -particles and 1 keV electrons this time is only  $10^{-9}$  sec and  $6 \times 10^{-9}$  sec respectively and hence the radical column will spread only very short distances before recombination can occur. The radicals from 60 keV electrons have a half-life of  $2 \times 10^{-6}$  sec, during which they can diffuse large distances

is not likely to be significant as the hydroxyl ions are initially distributed over a much larger volume than the hydrogen ions. Diffusion will bring about a spread of the two columns until at some given time they overlap completely (see *Figure 6* and *Table III*). For 60 kV x-rays no separate values are given for the concentration of the H and OH ions in *Table III*, as their columns already overlap along the tracks of the electron.

From simple kinetic considerations the number of collisions made

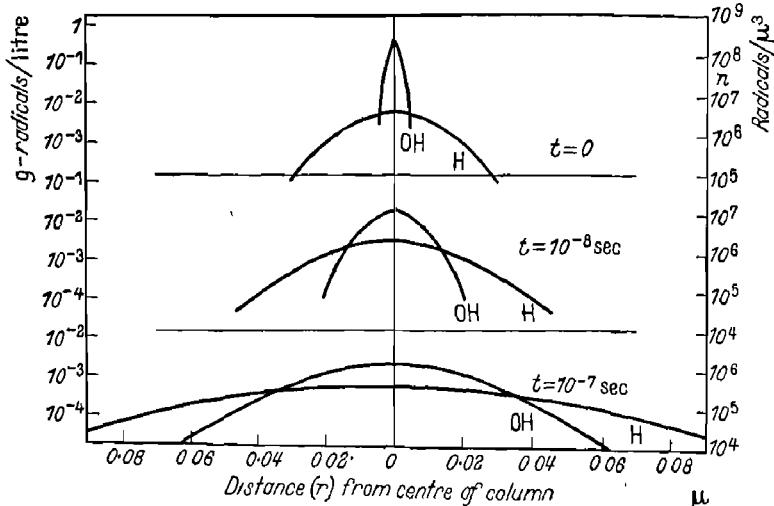
## SPATIAL DISTRIBUTION OF THE IONS FORMED BY DIFFERENT RADIATIONS

by radicals can be calculated, and in *Table III* the values for the interval between the formation of radicals and the time at which half of these have made one collision are given for different radiations. On the reasonable assumption that combination occurs at

*Table III.* Initial Distribution of Radicals formed by different Radiations in Water and their Diffusion<sup>19</sup>

	$\alpha$ -particle		1 kV electron		60 kV electron	
	$H_2O^+$ or $OH^-$	$H_2O^-$ or $H^-$	$H_2O^+$ or $OH^-$	$H_2O^-$ or $H^-$	$H_2O^+$ or $OH^-$	$H_2O^-$ or $H^-$
Mean separation of primary positive ions ( $\mu$ )	$8 \times 10^{-4}$	—	$5 \times 10^{-3}$	—	$9 \times 10^{-2}$	—
Radius of column ( $\mu$ )	$8 \times 10^{-4}$	$1.5 \times 10^{-2}$	$5 \times 10^{-3}$	$1.5 \times 10^{-2}$	$1.5 \times 10^{-2}$	—
Initial concentration of radicals (M)	1.0	$8.7 \times 10^{-3}$	$1.2 \times 10^{-2}$	$1.35 \times 10^{-3}$	$7.3 \times 10^{-5}$	—
Interval after which 50% of radicals will have made one collision with another radical (sec)	$10^{-11}$	$10^{-9}$	$7 \times 10^{-10}$	$6 \times 10^{-9}$	$1.6 \times 10^{-6}$	—
Expansion of column radius during this interval	1.06	1.02	1.11	1.11	7.6	—
Concentration of radicals at the end of the interval (M)	0.9	$8.4 \times 10^{-3}$	$1.0 \times 10^{-2}$	$1.1 \times 10^{-3}$	$1.3 \times 10^{-6}$	—

\* Primary positive ions only are considered in the case of  $\alpha$ -radiation. In the case of 1 kV electrons the positive column radius is large enough to include secondary positive ions.



*Figure 6.* Diffusion of the free radicals (according to LEA<sup>18</sup>) formed by  $\alpha$ -particle tracks in water

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

every collision between radicals this represents the time in which 50 per cent of the radicals have been converted to molecular products (*i.e.* H<sub>2</sub>, H<sub>2</sub>O<sub>2</sub> or recombination to water). *Table III* shows that this time is extremely short for  $\alpha$ -rays and is complete before the columns have diffused to any appreciable distance. Chemical effects of free radicals produced by  $\alpha$ -rays (and to a lesser extent by all radiation having an ionization density greater than 200/ $\mu$ ) in aqueous solution will, therefore, be confined to the immediate vicinity of the tracks and there will be no significant intermingling of radicals from different tracks unless the dose rate is extremely high.

The position for radiations of low specific ionization is quite different. The rate of recombination is much lower, since the initial local concentration is much smaller and intermingling of tracks will occur before an appreciable amount of recombination has taken place (see *Figure 5*). The distribution of radicals produced by sparsely ionizing radiation can, therefore, be considered as uniform and kinetic treatments similar to those used for reactions in homogeneous solutions can be used\*.

*Influence of substrate concentration on yield*—Based on this model Lea computed the relative rates at which active radicals are lost by recombination and by reaction with a solute as the function of the concentration of the latter. *Figure 7* shows the theoretical curves to which the limited experimental data available can be fitted. The exact position of the curve relative to concentration depends mainly upon the rate of reaction of the substrate molecule with the free radical (*i.e.* the average number of collisions which a radical has to make with a substrate molecule before reaction occurs) and to a lesser extent on the molecular weight. Experiments with protective agents show that this rate varies greatly from compound to compound (Chapter 14). For the relatively few substances for which curves of relative yield against concentration have been constructed, the limiting concentration (*i.e.* where the yield becomes constant) of x-ray induced reactions varies widely†. For the small organic

\* This procedure has been applied to many different reactions produced by x- and  $\gamma$ -rays and self-consistent results have been obtained except for the polymerization of acrylonitrile<sup>43</sup> and the reduction of oxygen dissolved in water to give hydrogen peroxide<sup>44</sup>. According to DANTON the kinetics of these reactions can only be explained if the radicals are non-uniformly distributed. Since the two exceptions show other unusual features<sup>45</sup> it is not justified to reject the concept that for chemical purposes the distribution of the radicals produced by hard radiation can be considered as uniform.

† As the limiting yield is reached asymptotically no final concentration can be given. For the purposes of this discussion the limiting concentration has been chosen as the point where the curve flattens out; the yield at this point was at least 90 per cent of the maximum.

## SPATIAL DISTRIBUTION OF THE IONS FORMED BY DIFFERENT RADIATIONS

molecules studied by FRICKE and HART<sup>23</sup> the value varied from  $3 \times 10^{-4}$  M for formic acid to  $2 \times 10^{-2}$  M for oxalic acid while methyl alcohol and formaldehyde were intermediate at  $10^{-3}$  M. As the size of all these molecules is of the same order the different limiting value must be ascribed to variations in the rate of reaction. The low value of  $10^{-5}$  M for carboxypeptidase<sup>19</sup> can be ascribed to its high molecular weight. For compounds of intermediate size, such as riboflavin<sup>46</sup>, fluorescein<sup>47</sup> and ascorbic acid<sup>48</sup> the limiting values are  $2 \times 10^{-4}$  M,  $3 \times 10^{-4}$ ,  $3 \times 10^{-3}$  M respectively. The enhancing effect of oxygen on two chemical reactions<sup>49, 50</sup> and many biological reactions<sup>49</sup> produced by x-rays reaches a limiting value at a concentration of  $10^{-4}$  M, and this may represent the limiting concentration for the reaction giving  $\text{HO}_2$  radicals (see p. 104).

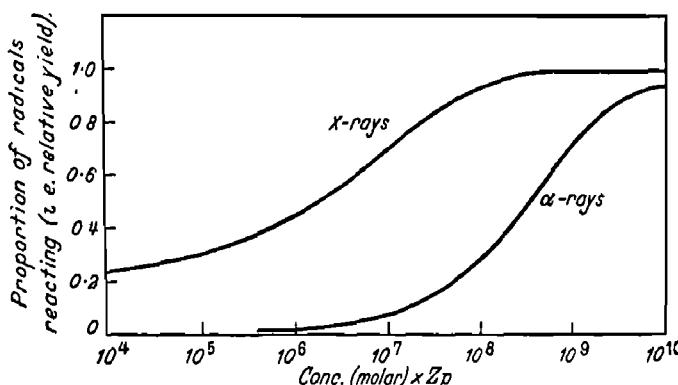


Figure 7. Influence of concn of substance dissolved in water on proportion of radicals utilized (based on Lea's model).  $Z$  and  $p$  are factors which determine efficiency of reaction between solute and radicals and which are independent of their respective concn

For  $\alpha$ -rays the experimental data for the number of free radicals produced is very limited. The only reaction treated in detail is the inactivation of carboxypeptidase<sup>19</sup>. This is a favourable case, since the enzyme is not affected by hydrogen peroxide so that a true estimate for the yield of radicals available for reaction with the solute can be obtained. The ratio of the ionic yield for  $\alpha$ - to x-rays was of the order of 0.04 and this value did not increase significantly with increase in concentration; the results did not indicate that the enzyme followed the theoretical curve of Figure 7 (i.e. that the same limiting M/N value though at much higher concentrations, should be reached for  $\alpha$ - as for x-rays). DALE, GRAY and MEREDITH<sup>19</sup> conclude that 'the small ionic yield observed for  $\alpha$ -rays is due principally to  $\delta$ -ray ionization and that the majority of the labile products have no opportunity of reacting with enzyme molecules because they are readily eliminated within the  $\alpha$ -ray column by

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

alternative reaction'. This deduction follows logically from the simple kinetic considerations that before a solute can react at all with the radicals it must be present at a concentration which is at least as great as that of the radicals in the columns surrounding the ionizing particle. For effective competition with the radicals the solute concentration will have to be many times greater. With 60 kV electrons (cf. *Table III*) the radical concentration is  $10^{-6}$  M and the concentration at which the ionic yield attains a limiting maximum value (*i.e.* when the solute molecules compete effectively for all the radicals) occurs at about  $10^{-4}$  M. For  $\alpha$ -particles the concentration of the OH radicals is molar and that of the H atoms  $10^{-2}$  M so that the concentration of the substrate would have to be several times molar before it could compete with radical recombination. In practice, therefore, the radicals never take part in reaction.

For the deamination of glycine the ratio of  $\alpha$ -ray to x-ray yield is higher at 0.15, but this reaction is complicated since it probably involves a chain process (see p. 113). The  $\alpha$ -ray yield is much larger for reactions in which hydrogen peroxide can take part. By measuring the amount of hydrogen involved, the contribution of the forward reaction to the oxidation of ferrous ions and of formic acid could be determined and the free radical yield was then found to be 20 per cent of that observed with x-rays (*i.e.* for  $\alpha$ -rays  $G_R = 0.6$  and  $G_F = 3$  to 4)<sup>51</sup>. The exceptionally low efficiency of  $\alpha$ -rays for inactivating carboxypeptidase is difficult to explain, but it must be remembered that the yield even with x-rays is unaccountably low, having a 'G' value for inactivation of 0.56.

*The B.M.S. theory*—The Lea treatment does not provide for a forward reaction giving molecular products the quantity of which is *initially* independent of the presence or concentration of a substrate. According to Lea the proportion of radicals which combine to give molecular products depend on the concentration and nature of the substrate. The quantitative development of the B.M.S. theory (see p. 85) by SAMUEL and MAGEE<sup>30</sup> directly provides for a forward reaction. Only those radicals which escape from the immediate vicinity of the track can react with a substrate. Formally this postulate appears to be similar to the conclusion reached by DALE *et al.*<sup>19</sup> that no reaction occurs within the track of a particle and that only the radicals from the  $\delta$ -rays can react. The important difference is that the Samuel and Magee treatment requires *both* radicals to be formed within the track of the ionizing particle whereas the Dale treatment applies only to OH radicals.

The fraction of the radicals which escape depends, according to the B.M.S. theory, on the ion density and the ratio of  $G_R$  for  $\alpha$ -rays

## SPATIAL DISTRIBUTION OF THE IONS FORMED BY DIFFERENT RADIATIONS

to  $G_R$  for x-rays calculated is 0.14; this value will be lower if the limiting concentration of the substrate has not been reached.

At the present time there is insufficient data to decide between the two theories. As we have seen, the local concentration of the radicals from  $\alpha$ -particles is so great that according to the Lea theory radical recombination will in practice always be the predominant reaction. For  $\alpha$ -rays the difference between the two theories is not significant. In the Lea model we have a core of OH radicals at approx. 1 M, and an outer cylinder of H atoms of  $10^{-2}$  M. Both radicals are present in such high concentrations that in practice no substrate could compete with radical recombination. For example, absence of an oxygen effect (*e.g.* in the production of  $H_2O_2$ ) with  $\alpha$ -particles may simply be due to the fact that the concentration of oxygen at ordinary pressures (concentration  $4 \cdot 10^{-4}$  M) is too low for it to capture any H atoms (present at  $10^{-2}$  M) to produce  $HO_2$  radicals. Exactly the same behaviour would be expected from the B.M.S. model in which H and OH radicals are present at the same concentration within the track. Whether the low  $G_R$  value observed is due entirely to radicals within the tracks of the  $\alpha$ -particles or to a small fraction of radicals which can escape from the track, is probably relatively unimportant. Both models suggest that for indirect processes where RBE of  $\alpha$ -rays is high (perhaps the breaking of chromosomes) the chemical changes are brought about by the local high (*i.e.* 1 M) concentration of  $H_2O_2$  which swamps the organisms' protective measures (*e.g.* catalase). Hydrogen peroxide at these concentrations would almost certainly bring about major chemical changes.

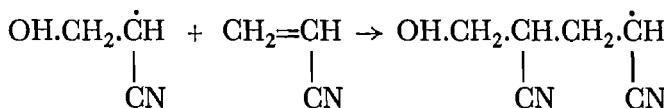
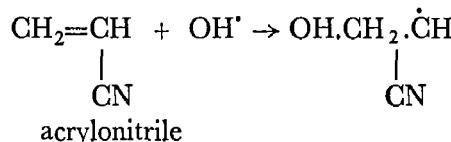
The two theories will show a significant difference for radiation of intermediate ionization (*e.g.* 5 kV electrons). In the Lea model the local concentration of OH radicals is then sufficiently high for recombination to predominate while a solute can compete for the H atoms; *i.e.* the amount of hydrogen and hydrogen peroxide will not be equal and the  $G_R$  for reaction with H (respectively  $HO_2$ ) will be greater than the  $G_R$  for reaction with OH. No such differences are to be expected if the B.M.S. model is operative.

## REACTION IN AQUEOUS SYSTEMS IN THE ABSENCE OF DISSOLVED OXYGEN

*Reactivity of the OH radical*—The principal evidence for the formation of OH radicals by  $\gamma$ - and x-rays is derived from the polymerization experiments of Dainton and his colleagues. The most effective way of polymerizing vinyl compounds in solution is by energetic

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

free radicals\* and the existence of such radicals can readily be detected in this way. Acrylonitrile dissolved in water was shown by DAINTON<sup>15</sup> to polymerize on irradiation with  $\alpha$ - or  $\gamma$ -rays. The reaction occurs as follows:



This active radical continues to add on monomer (*i.e.* acrylonitrile) until it is terminated by reacting with another radical. The initiating radical was proved to be OH since hydroxyl radicals could be detected spectroscopically in the polymer.

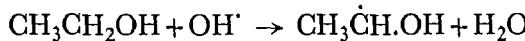
OH radicals can be produced chemically without ionizing radiations and the two most useful reactions† for their preparation are (i) irradiation of solutions of hydrogen peroxide with ultra-violet light or (ii) from a mixed oxidation-reduction system, the one most commonly used being ferrous sulphate and hydrogen peroxide.

- (i)  $\text{H}_2\text{O}_2 \xrightarrow{\text{u.v.}} 2\text{OH}^\cdot$
- (ii)  $\text{H}_2\text{O}_2 + \text{Fe}^{++} \rightarrow \text{OH}^\cdot + \text{Fe}^{+++} + \text{OH}^-$  (Fenton's reagent)

By studying the reactions of OH radicals prepared by these methods their chemical reactivity is now well understood<sup>52, 53</sup> and many of the chemical changes brought about by ionizing radiations in pure water can be reliably interpreted as OH radical reactions. These include:

- (1) Oxidation *e.g.*  $\text{Fe}^{++} + \text{OH}^\cdot \rightarrow \text{Fe}^{+++} + \text{OH}^-$

- (2) Abstraction of a hydrogen atom *e.g.*



- (3) Substitution at a double bond.

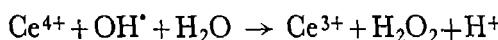
- (4) Reduction of powerful oxidizing agents.

\* Simple radicals such as H or OH are sufficiently reactive, but some complex organic radicals are stabilized by resonance and cannot initiate polymerization.

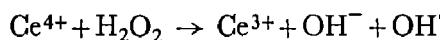
† In all methods of preparing OH radicals from peroxides HO<sub>2</sub> radicals will also be produced by the reaction  $\text{H}_2\text{O}_2 + \text{OH} \rightarrow \text{HO}_2 + \text{H}_2\text{O}$  and a mixture of the two is therefore produced in these chemical systems.

## REACTIONS IN ANAEROBIC AQUEOUS SYSTEMS

This last type of reaction needs amplification. The OH radical by virtue of its high electron affinity is a powerful oxidizing agent, but in the presence of other strong oxidizing agents it can be reduced. The reducing action was first demonstrated by FRICKE and BROWNSCOMBE<sup>54</sup> and has been studied in great detail by HAISSINSKY and his colleagues<sup>55</sup>. A typical example is the reduction of ceric sulphate



All these reactions are complicated and the final yield depends upon subsequent reactions. The behaviour of the OH radical in these reduction reactions is very similar to that of hydrogen peroxide *e.g.*

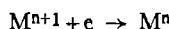


This probably explains why the yield from  $\alpha$ -rays in these cases is similar to that of x-rays.

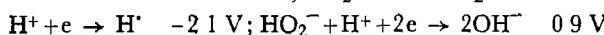
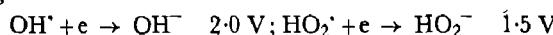
*Reactivity of the H atoms*—A notable feature of the radiation chemistry of aqueous solutions repeatedly stressed by HAISSINSKY<sup>55</sup> is the great preponderance of oxidative reactions, and in general an equilibrium condition is not set up in oxidative reactions.

The exact contrary would be expected if ionizing radiation produced, as is commonly assumed, both OH radicals and H atoms. The latter are powerful reducing agents and the preponderant radiochemical reactions would be reductions. The redox potential\* of an equimolecular mixture of H<sup>·</sup> and OH<sup>·</sup> would be 0.37 V. This means that only systems having a redox potential less than 0.37 V would be oxidized, all others being reduced. In *Table IV* a representative collection of x-ray-induced reactions in oxygen-free solutions is shown and from these it can be seen that only systems having a redox greater than about 0.9 V are reduced. Oxygen-free water irradiated with x-rays does, therefore, behave as a much more

\* The redox potential is a measure of the electron affinity of the substance undergoing reduction. Formally, reversible oxidation-reduction processes may be written



If into a solution containing an equi-molar mixture of M<sup>n</sup> and M<sup>n+1</sup> a reagent (*e.g.*, a free radical) is introduced with a redox potential greater than that of the initial system M<sup>n</sup> will be oxidized to M<sup>n+1</sup> (*i.e.* the radical will be reduced). Conversely, if the redox of the radical is less than that of the initial system M<sup>n+1</sup> will be reduced to M<sup>n</sup> and the radical will be oxidized. Values for the radicals concerned are given



The definition given here uses the British sign convention. The American convention is the exact opposite *e.g.* OH<sup>-</sup> → OH + e - 2 V.

### THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

powerful oxidizing agent than an equal mixture of the H and OH radicals. DANTON<sup>56</sup> points out that an inequality of H and OH could not resolve this difficulty since to change the equivalent redox potential of irradiated water from 0.37 to 0.9 V to ratio of OH to H would have to be of the order of  $10^{10}$  (*i.e.* effectively a complete absence of H).

*Table IV. Oxidizing and Reducing Action of x-ray and  $\gamma$ -rays. Influence of Redox Potential of System on the Nature of the Reaction<sup>57</sup>*

System	Redox potential (V)	Reaction	
Glutathione . .	-0.45	$2-\text{SH} \rightarrow -\text{S.S.}-$	oxidation
Cysteine . .	-0.27	$2-\text{SH} \rightarrow -\text{S.S.}-$	"
Ascorbic acid . .	-0.24	dehydro form	"
Formic acid . .	-0.14	$\text{HCOOH} \rightarrow \text{CO}_2 + 2\text{H}^+$	"
Iodide . .	0.53	$2\text{I} \rightarrow \text{I}_2$	"
Ferrous salt . .	0.77	$\text{Fe}^{++} \rightarrow \text{Fe}^{+++}$	"
Bromine . .	1.07	$\text{Br} \rightarrow \text{Br}^-$	reduction
Iodate . .	1.2	$2\text{IO}_3^- \rightarrow \text{I}_2$	"
Ceric salt . .	1.6	$\text{Ce}^{++++} \rightarrow \text{Ce}^{++-}$	"

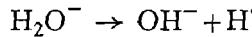
The concept of H and OH radical formation must, therefore, be modified and it may be necessary to invoke the formation of a third radical. DANTON and COLLINSON<sup>17</sup> suggested that the predominantly oxidizing character of irradiated water could be produced by the strong oxidizing radical  $\text{HO}^+$ , the formation of which in water vapour has been shown with the mass spectrometer. In view of the extremely high redox which these authors calculate for the  $\text{HO}^+$  radical only 14 per cent of these need be present with the H and OH radicals to render the equivalent redox the mixture equal to that found for irradiated water.

Alternatively WEISS<sup>58</sup> has proposed that the powerfully reducing H atoms react with chemically inert protons to give an oxidizing entity,  $\text{H}_2^+(\text{H}^\cdot + \text{H}^+ \rightarrow \text{H}_2^+)$ . Apart from the improbability of such a reaction on chemical grounds\* it is impossible on kinetic grounds in all but strongly acid solutions. Since the predominantly oxidative character of x-rays is not pH dependent<sup>55</sup> it cannot be explained by the reaction between a hydrogen atom and ion in solution. In other systems, such as gaseous discharges, the existence of an  $\text{H}_2^+$  ion is well established and its stability has been theoretically predicted<sup>63</sup>.

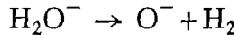
\* RIGG, STEIN and WEISS<sup>59</sup> claim that the influence of pH on the oxidation of ferrous sulphate can only be explained by the intervention of  $\text{H}_2^+$  ions. The validity of their experimental results has, however, been questioned by DEWHURST<sup>60</sup> and their theoretical treatments severely criticized by ABEL<sup>61</sup> and JOHNSON<sup>62</sup>.

### REACTIONS IN ANAEROBIC AQUEOUS SYSTEMS

HAISSINSKY and MAGAT<sup>64</sup> believe that H atoms are not formed on irradiation (or at least that they do not have an independent existence) and suggest that the  $\text{H}_2\text{O}^-$  ion does not break down as postulated by Lea.



but that the following process takes place:

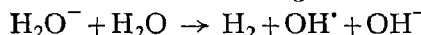


followed by  $\text{O}^- + \text{H}_2\text{O} \rightarrow \text{OH}' + \text{OH}^-$

or alternatively  $\text{H}_2\text{O}^- \rightarrow \text{H}' + \text{OH}'$

followed by  $\text{H}' + \text{H}_2\text{O} \rightarrow \text{H}_2 + \text{OH}^-$

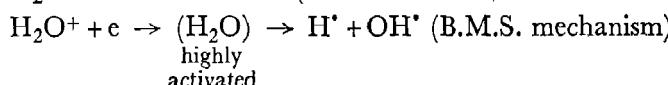
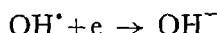
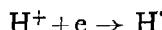
The overall reaction in both cases being



The energetics of these reactions have been considered by a number of authors, but no general agreement has yet been reached<sup>65</sup>. The formation of hydrogen molecules directly from  $\text{H}_2\text{O}^-$  seems difficult to reconcile with the postulate of the forward reaction giving equal quantities of  $\text{H}_2$  and  $\text{H}_2\text{O}_2$  which is well established experimentally (see p. 90).

Underlying all the discussion concerning the formation of H atoms<sup>96</sup> is the assumption that the 'thermal electron' cannot have an independent existence, but is captured within a time too short for it to undergo reactions with a solute by a water molecule or the positive ion from which it originated (see p. 85). The possibility that these electrons may have an independent existence of the order of  $10^{-5}$  sec, which is sufficient for reaction with dissolved materials cannot be excluded (see p. 86).

Thermal electrons with energy of considerably less than 1 V would be very feeble reducing agents (*i.e.* quite different from H atoms) and the predominantly oxidative character of irradiated pure water would be explained. These electrons could undergo a limited number of reactions but in general they would be captured by an atom having a high electron affinity (see p. 34) such as oxygen. In aerated water, therefore, the electrons would be quickly removed to give  $\text{O}_2^-$  (*i.e.*  $\text{HO}_2$ ). In pure water the electrons would eventually be lost by one of the following reactions:

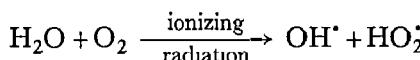


## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

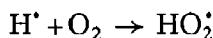
From the foregoing it is obvious that an essential feature is still missing in the understanding of the nature of 'activated' water produced by ionizing radiation. The only certainty is the existence of the OH radical. The simple concept, that the other radical is an H atom, cannot be reconciled with the chemical data. Fortunately, the position is considerably less confused in aerated solutions and it is these which are of primary interest to radiobiology.

### REACTIONS IN AERATED AQUEOUS SOLUTIONS

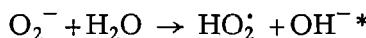
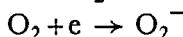
*Reactivity of the HO<sub>2</sub> radical*—The radical reaction in the presence of oxygen can be written with some confidence as



The HO<sub>2</sub> radical can be obtained from H atoms or from electrons

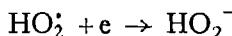


or



The HO<sub>2</sub> radical is believed to be less reactive and, therefore, more persistent than the OH radical<sup>67</sup>, and may play a role even in 'direct' action since every ionization process liberates an electron which, in the presence of oxygen, is converted to O<sub>2</sub><sup>-</sup> (*i.e.* HO<sub>2</sub>). The size of a target may, therefore, be extended since the HO<sub>2</sub> radical produced in an ionization in a non-vulnerable spot may diffuse and react with a vital centre. This is a special case of the general problem of energy transfer in relation to direct action which has been considered on p. 62. Attention is also focussed on the HO<sub>2</sub> radical by the great influence of oxygen on radiobiological effects (see Chapter 8) and by experiments with protection agents (see Chapter 14).

Little is known of the chemical reactivity of the HO<sub>2</sub> radical and how it differs from OH radicals. Like the latter it is an oxidizing agent, though somewhat less powerful (*i.e.* having a lower or more negative redox potential) and readily captures an electron.

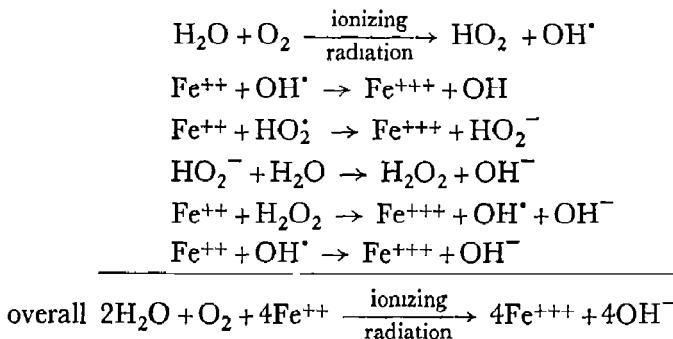


HO<sub>2</sub><sup>-</sup> is the anion H<sub>2</sub>O<sub>2</sub> (a very weak acid of pk 11) and in all except strongly alkaline solutions the process HO<sub>2</sub><sup>-</sup> + H<sup>+</sup> → H<sub>2</sub>O<sub>2</sub>

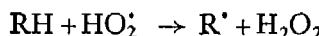
\* This step is automatic as HO<sub>2</sub> dissociates as an acid and the pH of the solution governs the position of this equilibrium. Unfortunately the pk of the HO<sub>2</sub> radical is not known<sup>66</sup>; there is some evidence that it is a moderately strong acid of pk 3 while other facts suggest that the pk is much higher and of the order of pk 10. Depending on the value chosen therefore, the radical will be either wholly in the ionized form or wholly undissociated under physiological conditions.

REACTIONS IN AERATED AQUEOUS SOLUTIONS

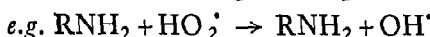
takes place immediately. After reduction, therefore, the  $\text{HO}_2$  radical is converted into hydrogen peroxide, which can oxidize suitable substrates further. The  $\text{HO}_2$  radical can be considered to react in two stages. In the first it is a powerful oxidizing agent of redox potential 1.5 V capable of oxidizing one equivalent and in the second a much less powerful reagent of redox 0.9 V capable of oxidizing two equivalents. The total oxidizing capacity of  $\text{HO}_2$  is three equivalents. For each molecule of water decomposed by ionizing radiation in the presence of air a total of four oxidizing equivalents are produced. The oxidation of ferrous sulphate in aerated acid solutions can be written as follows:



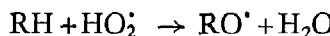
Besides oxidation,  $\text{HO}_2$  radicals can probably, like OH radicals, abstract hydrogen atoms from suitable organic substances (RH).



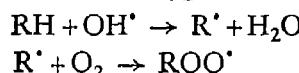
In addition the possibility that  $\text{HO}_2$  can donate an oxygen atom to a molecule containing a lone pair of electrons has been suggested<sup>68</sup>



The exchange reaction



may explain the superiority of  $\text{HO}_2$  over OH radicals in degrading macromolecules (*cf.* p. 132) since RO radicals are known to be very unstable and to decompose further. An OH radical in the presence of oxygen could abstract a hydrogen atom from the organic molecule which could then add an oxygen molecule as follows:



The ROO radical produced in this way is much less likely to disproportionate than the RO radical.

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

The potentiating effect of oxygen on the x-ray induced oxidation of ferrous sulphate<sup>49</sup> and the degradation of polymethacrylic acid<sup>50</sup> becomes detectable at an oxygen concentration of about  $10^{-6}$  M; below this value the solution behaves as if it were completely anaerobic. The limiting value (see p. 96) for the oxygen effect occurs at about  $10^{-4}$  M (see *Figure 8*) which is of the same order of magnitude as the concentration of oxygen in pure water, which is in

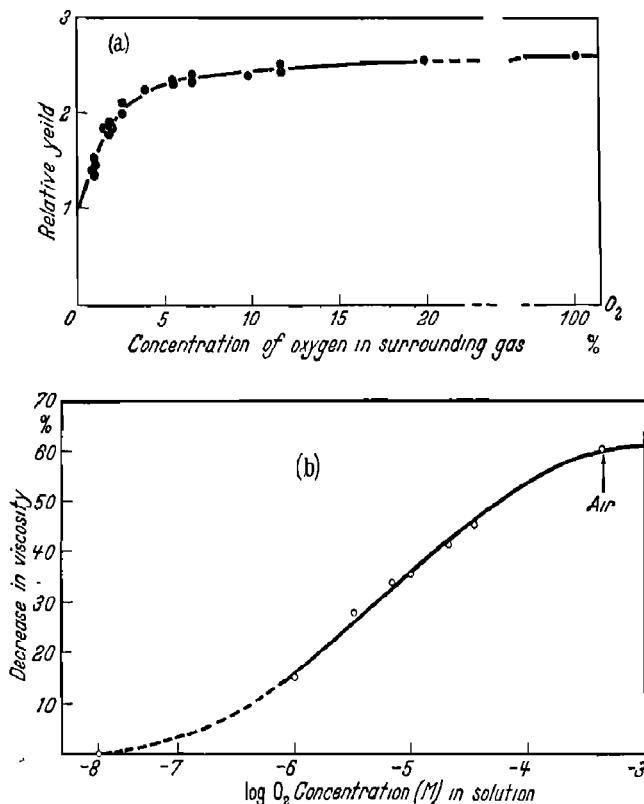


Figure 8(a). Increase in the ionic yield for the oxidation of ferrous sulphate as the oxygen concentration in the solution is increased from nothing to saturation<sup>49</sup>. (b) Influence of concentration of dissolved oxygen on the degradation of polymethacrylic acid<sup>50</sup>

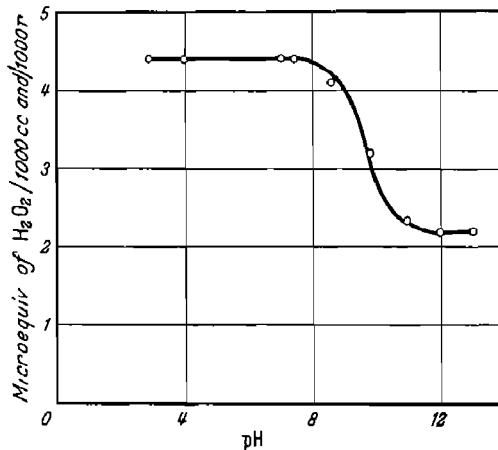
equilibrium with air ( $4 \times 10^{-4}$  M). In this concentration range, therefore, oxygen reacts with all the available H atoms (or electrons) to give a quantitative conversion to  $\text{HO}_2$  radicals.

The amount of ferrous ion oxidized by  $\alpha$ -rays is approximately the same in solution saturated with oxygen as in solutions under nitrogen and no  $\text{HO}_2$  radicals would appear to be formed. This is to be expected since the concentration of H atoms in the track is too

great for the oxygen in aerated water to compete with the radical recombination process giving hydrogen molecules. The oxidizing capacity is due to molecular hydrogen peroxide (see p. 89). A contribution to the effect of  $\alpha$ -rays (possibly as much as 20 per cent) is believed to be made by free radicals at lower concentrations (*i.e.* in  $\delta$ -ray tracks or by 'escape' from the main track) and this part should be potentiated by oxygen although this has not been detected. There appears to be no published information about the effect of oxygen on the chemical reactions produced by radiations with specific ionizations intermediate between those from therapy x-ray sets and 5 MeV  $\alpha$ -particles.

*Formation of hydrogen peroxide*—Following the earlier discovery<sup>11, 12</sup> that pure water is not decomposed by x-rays (100 kV), FRICKE<sup>69</sup>

Figure 9. Influence of pH of the solution on the formation of hydrogen peroxide in aerated water by x-rays<sup>69</sup>



showed that oxygen dissolved in water was reduced to hydrogen peroxide. The yield of the reaction was independent of oxygen concentration over a wide range, was greater at low pH values (see Figure 9) and was reduced in the presence of simple salts such as sodium chloride (see Table V). A number of workers prominent in this field, by co-ordinating their techniques, have been able to publish an agreed statement of these experimental results (see Figure 10) which are now beyond dispute<sup>70, 44</sup>. The notable features are (i) the amount of peroxide produced tends towards a limiting value indicating an important back reaction; (ii) in acid solution (the change occurring between pH 2 and 6) both the initial rate of formation as well as the limiting value attained are much greater than in neutral solution; (iii) under comparable conditions

THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

30 and 200 kV x-rays (both having similar average specific ionizations) produce hydrogen peroxide at a greater rate than 1 MeV electrons; the 'G' values based on initial rates being 2.2 and 1.05 respectively. Although there is a tenfold difference in specific ionizations between these radiations this is the first definite example of a chemical difference between radiation in this range of ion densities. On theoretical grounds (see p. 96) one would not expect this difference since the radicals can diffuse to give an essentially homogeneous distribution before significant recombination has occurred.

The influence of the oxygen concentration on the formation of hydrogen peroxide is also unusual<sup>49, 69</sup>. The initial rate rises

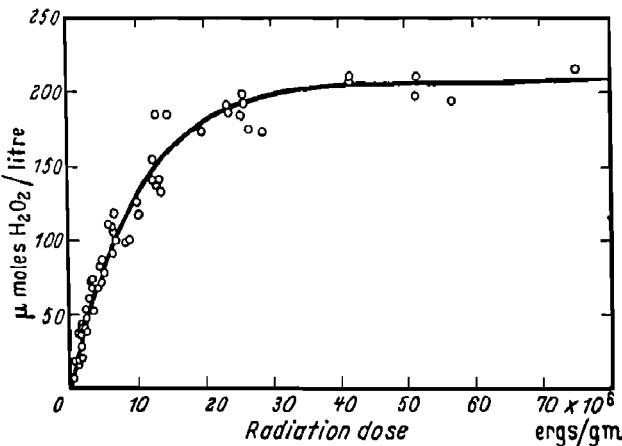
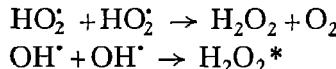


Figure 10. Relationship between radiation dose (1.2 MeV x-rays) and quantity of  $\text{H}_2\text{O}_2$  formed in aerated water<sup>70</sup>

sharply at very low concentration of oxygen and has already reached its maximum value at  $10^{-5}$  M (*i.e.* when the solution is in equilibrium with gas containing 0.5 per cent of oxygen). This behaviour is quantitatively different from the influence of oxygen on other x-ray induced reactions. A further surprising feature is that the limiting or equilibrium concentration of the hydrogen peroxide produced is directly proportional to the oxygen concentration.

No reaction scheme has yet been put forward which can account for all these phenomena. The principal reactions leading to the formation of hydrogen peroxide are believed to be<sup>44</sup>

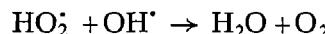



---

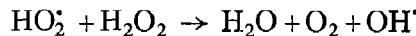
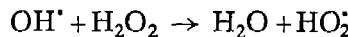
\* At one time objections were raised against this reaction, but it is now believed to occur readily in aqueous systems.

### REACTIONS IN AERATED AQUEOUS SOLUTIONS

Competing with these reactions will be the radical removal reaction



When appreciable quantities of hydrogen peroxide have been formed the following process may bring about the equilibrium state:



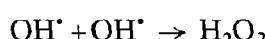
The last process is relatively slow and the OH radical decomposition will predominate<sup>72</sup>.

*Influence of added substances on hydrogen peroxide formation*—In the absence of oxygen most substances capable of reacting with OH radicals will allow the accumulation of hydrogen peroxide formed by the molecular 'F' reaction by suppressing the back reaction (see p. 87). In aerated solutions the radical reaction leads to hydrogen peroxide formation and added substance may promote or retard this radiochemical reaction (see *Table V*). Substances

*Table V. Influence of Added Substances on the Amount of Hydrogen Peroxide formed in Aerated Water on Irradiation with x-rays*

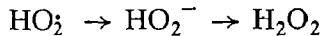
Solute	H <sub>2</sub> O <sub>2</sub> formed in solution	Reference
	H <sub>2</sub> O <sub>2</sub> formed in pure water	
Formic acid 10 <sup>-3</sup> M . . .	1.7	23
Formic acid 3 × 10 <sup>-5</sup> M . . .	1.5	23
Hydroquinone 10 <sup>-3</sup> M . . .	1.9	93
Hydroquinone 10 <sup>-5</sup> M . . .	1.3	93
Cysteine 10 <sup>-3</sup> M . . .	1.9	93
Cysteine 10 <sup>-5</sup> M . . .	1.3	93
Ascorbic acid 10 <sup>-3</sup> M . . .	3.1	93
Ascorbic acid 10 <sup>-5</sup> M . . .	1.1	93
Benzene . . .	2.8	94
Sodium chloride 0.5 M . . .	0.6	95
Sodium nitrate 0.5 M . . .	0.06	95
Sodium nitrate 0.1 M . . .	0.12	95
Crotonic acid 10 <sup>-2</sup> M . . .	0.75	93
Crotonic acid 10 <sup>-5</sup> M . . .	1.1	93
Succinic acid 10 <sup>-2</sup> M . . .	0.4	93
Succinic acid 10 <sup>-5</sup> M . . .	1.0	93
Fumaric acid 10 <sup>-2</sup> M . . .	0.5	93
Fumaric acid 10 <sup>-5</sup> M . . .	1.1	93

which react readily with OH radicals will lower the yield by decreasing the reaction

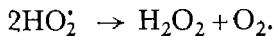


THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

Additives which are oxidized by  $\text{HO}_2^\cdot$  to form  $\text{HO}_2^-$  will increase the yield since one molecule of  $\text{H}_2\text{O}_2$  is then obtained per  $\text{HO}_2^\cdot$  radical, i.e.



instead of



Since the  $\text{H}_2\text{O}_2$  yield is increased by the addition of benzene, ascorbic acid, hydroquinone and cysteine it would appear that these

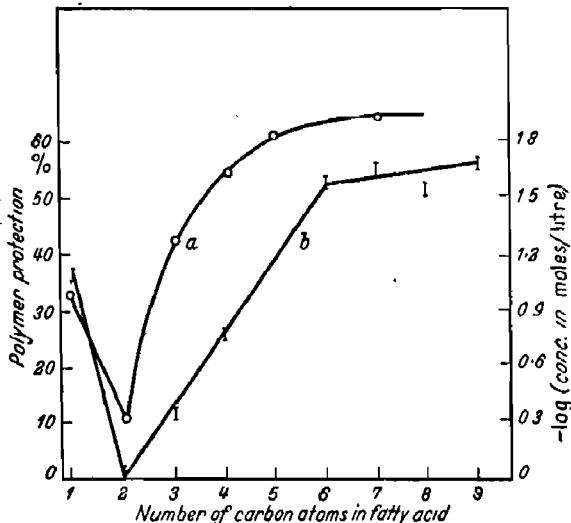


Figure 11. Reactivity of aliphatic fatty acids with  $\text{HO}_2^\cdot$  radicals.  
 (a) Curve shows relationship between concentration of acid required to double the amount of  $\text{H}_2\text{O}_2$  formed by irradiating aerated water with  $\text{x}$ -rays and the constitution of the acid (concn is expressed as  $-\log$  of the molarity; consequently as the effectiveness of the acids increases the concn required becomes less and the  $-\log$  of the concn becomes more negative)<sup>54</sup>. (b) Curve shows their effectiveness as protective agents. Polymethacrylic acid is degraded by  $\text{HO}_2^\cdot$  radicals and these acids compete with the polymer for these radicals. The percentage protection conferred by a given concn of fatty acid is a measure of its reactivity with  $\text{HO}_2^\cdot$  radicals<sup>73</sup>

are reduced more rapidly by  $\text{HO}_2^\cdot$  than  $\text{OH}^\cdot$  radicals; if they reacted equally readily the amount of  $\text{H}_2\text{O}_2$  formed would be unchanged. Conversely, the substances which reduce the yield can either react more readily with  $\text{OH}^\cdot$  radicals or combine with  $\text{HO}_2^\cdot$  radicals in a way which does not give  $\text{HO}_2^-$  or  $\text{H}_2\text{O}_2$  (*cf.* p. 105). These deductions are supported from studies on protection (Chapter 14) when, for example, benzene was shown to protect against  $\text{HO}_2^\cdot$  but not against  $\text{OH}^\cdot$  reactions. More impressive is the parallelism shown

#### REACTIONS IN AERATED AQUEOUS SOLUTIONS

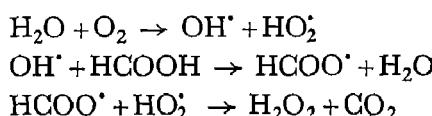
in *Figure 11* between the potentiating effect of a series of aliphatic acids on hydrogen peroxide formation by x-rays<sup>54</sup> and the protection by the same acids against the degradation of polymethacrylic acid by x-ray produced HO<sub>2</sub> radicals<sup>73</sup>. Both reactions can be interpreted in terms of the preferential reduction of fatty acids in the order shown by HO<sub>2</sub> radicals to give HO<sub>2</sub><sup>-</sup> (*i.e.* H<sub>2</sub>O<sub>2</sub>).

Added substances do not influence the yield of hydrogen peroxide by  $\alpha$ -particles which is also independent of oxygen concentration<sup>49</sup>. These results are in agreement with other observations, that the large majority of the radicals formed by  $\alpha$ -rays are not accessible to dissolved substances even when these are present in high concentration and support the view of GRAY<sup>20</sup> that some at least of the *in vivo* effects of  $\alpha$ -rays can be ascribed to the formation of H<sub>2</sub>O<sub>2</sub> at high concentration (about 1 M).

#### REACTIONS OF ORGANIC SUBSTANCES DISSOLVED IN WATER

The first systematic experiment in this field was carried out by KAILAN<sup>74, 75</sup>, who found that many organic substances were changed when irradiated in aerated aqueous solutions with the mixed  $\beta$ - and  $\gamma$ -rays from radium. M/N values quoted varied from 0.2 for cane sugar to 1.5 for succinic acid. Our detailed knowledge in this field comes from the very important work of HART and FRICKE<sup>23</sup> who carried out detailed kinetic studies on the effect of x-rays on many simple aliphatic compounds dissolved in oxygen-free water. In most of the reactions the yield was proportional to dose and independent of initial concentration once a certain minimum was passed. Some representative results are summarized in *Table VI*.

The reaction of formic acid with x-rays was also studied in solutions containing oxygen when the whole course of the reaction was changed, as shown in *Table VII*. HART<sup>76</sup> has extended his original work with Fricke and suggests that the reaction with oxygen occurs as follows:



The principal reaction of x-rays on amino-acids dissolved in water is deamination and is not influenced by the presence of oxygen nor

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

 Table VI. The effect of x-rays on Simple Organic Molecules dissolved in Daeerated Water<sup>23</sup>

Main reaction	pH	'G' value*
$\text{CO} \rightarrow \text{CO}_2 + \text{H}_2$ (+ some formaldehyde)	1.7	3.9 ( $\text{H}_2$ and $\text{CO}_2$ )
$\text{HCOOH} \rightarrow \text{H}_2 + \text{CO}_2$	3	3.2 ( $\text{H}_2$ and $\text{CO}_2$ )
$2\text{HCOOH} \rightarrow (\text{COOH})_2 + \text{H}_2$ (formic acid)	7	3.7 ( $\text{H}_2$ )
$\text{CH}_3\text{COOH} \rightarrow \text{CH}_2(\text{OH})\text{COOH} + \text{H}_2$ (acetic acid)	3	3 ( $\text{H}_2$ ) At high concn some $\text{CO}_2$ is obtained
$\text{CH}_3\text{CH}_2\text{COOH} \rightarrow ? + \text{H}_2$ (propionic acid)	3	2.5 ( $\text{H}_2$ )
$\text{CH}_3(\text{CH}_2)_2\text{COOH} \rightarrow ? + \text{H}_2$ (butyric acid)	3	2.0 ( $\text{H}_2$ )
$(\text{COOH})_2 \rightarrow ? \text{CO}_2 + \text{H}_2$ (oxalic acid)	3	3.6 ( $\text{CO}_2$ ) This applies to $10^{-4}$ M solns; as concn is increased more $\text{CO}_2$ and less $\text{H}_2$ is obtained
Maleic $\rightarrow ? + \text{H}_2$	3	2.5 ( $\text{H}_2$ )
Succinic $\rightarrow ? + \text{H}_2$	3	2.5 ( $\text{H}_2$ )
$\text{HCHO} \rightarrow \text{HCOOH} + \text{H}_2$ (formaldehyde)	4	3 ( $\text{H}_2$ ) Reaction more complex in 3 ( $\text{HCOOH}$ ) alkali
$\text{CH}_3\text{CO.H} \rightarrow \text{CH}_3\text{COOH} + \text{H}_2$ (acetaldehyde)	4	1.1 ( $\text{H}_2$ )
$\text{CH}_3\text{CO.CH}_3 \rightarrow ? + \text{H}_2 + \text{H}_2\text{O}_2$ (acetone)	3	1.3 ( $\text{CH}_3\text{COOH}$ )
	3	1.8 ( $\text{H}_2$ )
	3	0.4 ( $\text{H}_2\text{O}_2$ )

\* For product shown in brackets

 Table VII. Influence of Oxygen on the Decomposition by x-rays (1000 r) of Formic Acid in Aqueous Solution<sup>23</sup>

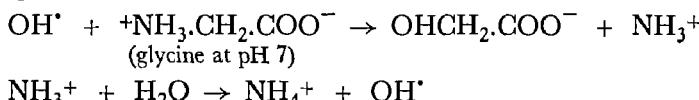
Concentration of formic acid	Products produced (micromoles)					
	Gas free solution		Oxygenated solution*			
	$\text{CO}_2$	$\text{H}_2$	$\text{CO}_2$	$\text{H}_2$	$\text{H}_2\text{O}_2$	
$1.00 \times 10^{-3}$ M	.3.2	.3.2	.2.1	.0.8	.3.4	
$0.03 \times 10^{-3}$ M	.2.7	.2.7	.1.6	.0.5	.3.2	
None	—	—	—	—	—	.2.2

\* In equilibrium with oxygen at 6 cm (Hg) pressure

by the addition of hydrogen peroxide; this indicates that OH radicals play a predominant part. The most unusual feature of the reaction is that the yield increases continually with concentration (see Figure 12) and does not reach a limit. DALE *et al.*<sup>77</sup> have dis-

## REACTIONS OF ORGANIC SUBSTANCES DISSOLVED IN WATER

cussed several possible explanations for this behaviour, and the most probable mechanism is a chain reaction as follows\*:



The deamination reaction is only given by  $\alpha$ -amino-acids and amino groups in other positions or in related compounds such as urea or thiourea do not give ammonia in high yield†. The ionic yield at a given concentration is the same for all the  $\alpha$ -amino-acids studied with the exception of histidine for which it is higher, and for

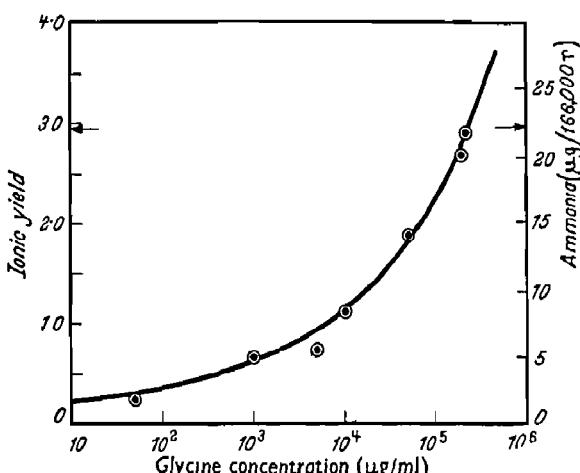


Figure 12. Evolution of ammonia on irradiating aqueous solutions of glycine with x-rays<sup>77</sup>. Relationship between ionic yield and concn of glycine (arrow gives the value when dry glycine is irradiated)

cysteine which is not deaminated. Colorimetric tests show that the imidazole ring is also attacked when histidine is irradiated<sup>78</sup>. Deamination of glycine by  $\alpha$ -rays is essentially similar to the x-ray induced reaction, except that the yield is much lower<sup>79</sup>.

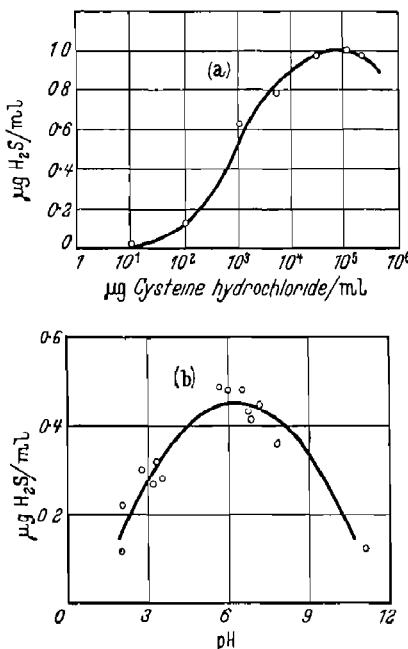
The reactions of cysteine and of glutathione are of particular importance in view of the interest which attaches to the inactivation of sulphhydryl enzymes (see p. 140). BARRON<sup>80</sup> claims that the only reaction which occurs is the oxidation  $2 \text{R.SH} \rightarrow \text{R.S.S.R.}$  and finds an ionic yield of one in the absence of oxygen and of four in its

\* Thiourea splits out sulphur on irradiation and like glycine the ionic yield increases with concentration to give very high ionic values. No mechanism has been put forward<sup>71</sup>.

† An apparent exception is *p*-aminobenzoic acid<sup>83</sup> which is both decarboxylated and deaminated when irradiated in aqueous solution.

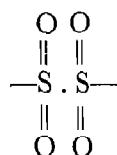
### THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

presence, in apparently perfect agreement with simple theory. Detailed work of DALE and colleagues<sup>82</sup> showed that the reaction is much more complicated. Hydrogen sulphide is formed, and the yield corresponds to a 'G' value of 0·9 at relatively high concentrations of cysteine. The yield varies with pH as shown in *Figure 13*.



*Figure 13. Evolution of H<sub>2</sub>S on irradiating aqueous solutions of cysteine with x-rays 82. (a) Influence of concn on yield (33,000 r at pH 2). (b) Influence of pH on yield (cysteine hydrochloride concn 5·10<sup>3</sup> μg/ml; dose 9500 r)*

The oxidation of the sulphydryl group also is not straightforward and in addition to the formation of disulphide bonds higher oxidations to sulphoxide



occur<sup>84</sup>. As a byproduct of the irradiation of cystine H<sub>2</sub>O<sub>2</sub> is produced (see *Table V*). No hydrogen sulphide is obtained on irradiating cystine which unlike cysteine is deaminated. From the foregoing it would appear that the excellent quantitative agreement of the experiments of BARRON<sup>80</sup> with simple theory may be fortuitous and that the oxidation to disulphide is only one of several reactions which sulphydryl compounds can undergo.

The behaviour of aromatic compounds is extremely complex and

## REACTIONS OF ORGANIC SUBSTANCES DISSOLVED IN WATER

relatively little detailed information concerning the actual processes is known. A typical reaction is the oxidation of benzene to phenol, first described by ERRERA and HENRI<sup>85</sup>, and studied more recently by STEIN and WEISS (see footnote p. 91). BOYLAND and SIMS<sup>86</sup> have studied the oxidation of a number of compounds including naphthalene by chemically produced OH and HO<sub>2</sub> radicals and isolated a number of different products.

All biologically active organic molecules which have been studied were inactivated when irradiated in aqueous solution by x-rays, although in few cases only (*e.g.* *p*-aminobenzoic acid<sup>83</sup>, nicotinamide, ascorbic acid<sup>48</sup>, riboflavin<sup>46</sup>, glutathione<sup>98</sup>) is anything known concerning the mechanism or the relative yields.

## INFLUENCE OF HIGH DOSE RATE ON RADIOCHEMICAL REACTIONS

When considering radical recombination processes it is in general only necessary to deal with radicals formed within the same track since the distance between tracks is much greater than the distance between ionization (or excitations) within the track. For this reason dose rate does not influence the number of primary chemical events. For example, MILLER<sup>87</sup> found that the 'G' value for the oxidation of ferrous sulphate was independent of dose rate up to the highest value tested of 100,000 r/min.

With extremely high dose rates this no longer applies; in generators, such as the betatron, synchrotron or with linear accelerators, the electrons (or the x-rays) are emitted over very short time intervals (of the order of 10<sup>-6</sup> sec). In these short pulses, dose rates of the order of 10<sup>6</sup> r/min may be attained, although the integrated dose rate will be much smaller as usually there are only 50 such bursts per second. The energy of the electrons emitted by these generators is usually of the order of several MeV and the specific ionization is, therefore, extremely low. Yet many chemical reactions have been studied, notably by HUBER<sup>88</sup> in which the radiochemical yield was smaller with a betatron than with 200 kV x-rays (see *Table VIII*). Free radical reactions (except chain reactions; see footnote p. 96) ought to be the same for all radiations of specific ionization less than 50/ $\mu$  since the separation between ionizations is then sufficiently great for the free radicals to diffuse away from the track before appreciable recombination has occurred. The probable explanation for the differences shown in *Table VIII* is that very high dose rates are temporarily produced by the electron

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

*Table VIII. Comparison of the Effects produced by High Intensity Bursts of 16 MeV Electrons with those brought about by 'therapeutic' (e.g. 200 kV) x-rays<sup>88, 89, 90</sup>*

<i>Effect produced by x-rays or continuous β-irradiation</i>		<i>Effect of high intensity bursts</i>
<b>CHEMICAL REACTIONS</b>		
Polymerization of styrene		No change even with high doses
Degradation of haemoglobin to give lower molecular weight products		No degradation but some methaemoglobin formed
Evolution of H <sub>2</sub> and CH <sub>4</sub> from butane and heptane		No change
<b>BIOLOGICAL EFFECTS</b>		
System		RBE (High intensity bursts: x or γ)
Escherichia coli		1.45
Vicia faba abnormal anaphases		0.8
Drosophila sex-linked lethal mutations		1
Mouse acute lethal dose		0.65
Mouse lowering of reticulocyte count		2.0

bursts. Consequently the number of ionizing particles passing through the irradiated volume is so large that the average concentration of free radicals is sufficiently high for recombination to occur\*.

No similar effect should be observed when the action of the radiation is direct as radical recombination can play no part and the very energetic betatron radiations should be more effective than, for example, x-rays from ordinary therapy sets, as fewer clusters will be formed (see p. 21). For a number of biological effects betatron radiations have been shown<sup>90</sup> to be more efficient than 20 kV x-rays, but insufficient detailed data is available to decide if the increased action can be explained in terms of a single-hit process.

### EFFECT OF RADIATION ON COLLOIDS

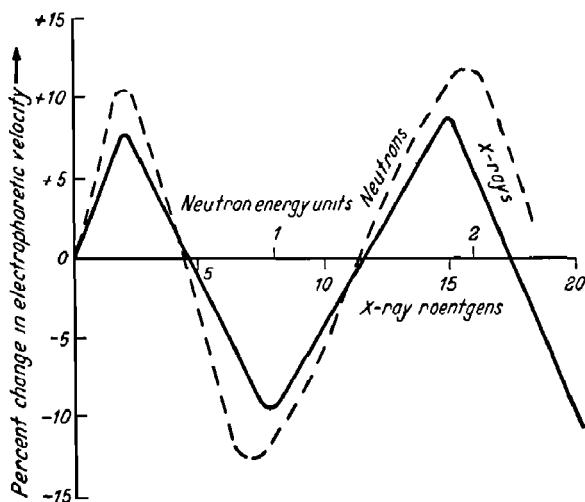
Besides producing chemical changes, ionizing radiations also alter the physical properties of aqueous colloids, but the mechanism by which these changes are brought about is not understood. CROWTHER and FAIRBROTHER<sup>91</sup> observed that irradiation of metal sols by soft x-rays produced precipitation if the particles carried a positive charge, but that anionic colloids were stabilized. If the dose of x-rays was not large enough to produce precipitation the colloid was nevertheless sensitized, and the quantity of a coagulating agent required for precipitation was reduced. The changes produced by radiation were found to be permanent and the amount of

\* The fact that betatron electrons do not polymerize vinyl polymers at all is probably due to the fact that the concentration of the free radicals throughout the solution exposed to the radiation is sufficiently high for chain termination reactions to prevent the propagation of the polymer chain (*cf.* COLLINSON and DANTON<sup>43</sup>).

### EFFECT OF RADIATION ON COLLOIDS

coagulating agent needed for precipitation was not dependent on the time interval which had elapsed between its addition and the irradiation.

A related effect of x-rays on colloids, also discovered by CROWTHER<sup>92</sup> is a change in the zeta (or electrokinetic) potential. Using a sol of graphite, small doses of x-rays (*i.e.* 10 to 100 r) were found sufficient to bring about an alternate increase and decrease in this property (see *Figure 14*) which was determined directly by measuring the rate of electrophoretic movements. As the dose is increased the alternation in zeta potential continues without any change in amplitude but the interval between peaks (*i.e.* the wavelength) increases. Crowther was well aware that this amazing effect was



*Figure 14. Effect of low doses of x-rays and fast neutrons on the electrophoretic behaviour of a colloidal sol* <sup>97</sup>

observed near the limit of experimental accuracy and took elaborate precautions to establish that the measurements were significant. The change in zeta potential is permanent and independent of the rate at which the dose was given (*i.e.* the first maximum occurs after 15 r whether this is given at 0.2 r/min or 2 r/min).

The zeta potential is a function of the charge on the surface of the colloid and the thickness of the so-called electric double layer\*, which depends almost entirely on the ionic strength of the solution and will, therefore, not be changed by irradiation. The factor

\* The electric double layer is the film of water surrounding suspended particles in which the distribution of the ions is not uniform and differs from that in the bulk of the solution.

## THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

most likely to be changed is the surface charge and it is conceivable that the ion pairs produced (or the thermal electron before it is captured to give the negative ion) may combine with the colloid. Although the dose is small only the surface of the particle has to be changed and a simple calculation shows that sufficient electrons were available in Crowther's experiments to bring about a detectable change in the surface charge and, therefore, in the zeta potential. No reason can be suggested why the change in charge should alternate.\*

These experiments provide a challenge, and it is surprising that they have not received more attention. Biologically, changes in zeta potential are of great interest for two reasons: (i) They are detectable at doses which in general are too low to produce a measurable chemical change, but which are nevertheless sufficient to produce pronounced biological effects; and (ii) cells are most radiosensitive at or near division when colloidal constituents of the nucleus, such as chromosomes, are undergoing changes, which are probably very sensitive to alteration in zeta potential. The possibility that purely physicochemical changes may be responsible for some of the observed effects of ionizing radiations has to be considered, although it will be difficult to test experimentally.

### REFERENCES

- <sup>1</sup> CURIE, P. and DEBIERNE, A., *C.R. Acad. Sci., Paris*, 1901, **132**, 770
- <sup>2</sup> RAMSAY, W. and SODDY, F., *Proc. Roy. Soc.*, 1903, **72**, 209
- <sup>3</sup> RAMSAY, W., *J. chem. Soc.*, 1907, **91**, 931
- <sup>4</sup> KIRNBAUM, M., *Radium, Paris*, 1910, **7**, 42
- <sup>5</sup> CAMERON, A. F. and RAMSAY, W., *J. chem. Soc.*, 1908, **92**, 966
- <sup>6</sup> BRAGG, W. M., *Phil. Mag.*, 1907, **13**, 333
- <sup>7</sup> LIND, S. C., *The Chemical Effects of Particles and Electrons*, New York, 1928
- <sup>8</sup> DUANE, W. and SCHEUER, O., *Radium, Paris*, 1915, **10**, 33  
SCHEUER, O., *C.R. Acad. Sci., Paris*, 1914, **159**, 423
- <sup>9</sup> KAILAN, A., *S. B. Akad. Wiss. Wien*, 1911, **120** (11a), 1213
- <sup>10</sup> KAILAN, A., *ibid.*, 1917, **128** (11a), 8713
- <sup>11</sup> FRICKE, H. and BROWNSCOMBE, E. R., *Phys. Rev.*, 1933, **44**, 240
- <sup>12</sup> RISSE, O., *Z. phys. Chem.*, 1929, **A140**, 133
- <sup>13</sup> RISSE, O., *Strahlentherapie*, 1929, **34**, 578
- <sup>14</sup> WEISS, J., *Nature*, 1944, **153**, 748
- <sup>15</sup> DAINTON, F. S., *J. phys. Colloid Chem.*, 1948, **52**, 490

\* Similar observations have been made in biological systems. Of particular interest is the detailed investigation by FORSSBERG<sup>71</sup> with the microspore *Phycomyces Blakesleeanus* in which extremely small doses of irradiation with x-rays temporarily altered the growth rate. The change alternated from increase to decrease as the dose was increased.

#### REFERENCES

- <sup>16</sup> MANN, M. M., HUSTRULID, A. and TATE, J. T., *Phys. Rev.*, 1940, **58**, 340
- <sup>17</sup> DAINTON, F. S. and COLLINSON, E., *Annu. Rev. phys. Chem.*, 1951, **2**, 108
- <sup>18</sup> LEA, D. E., *Brit. J. Radiol.*, Suppl. No. 1, 1947, p. 59
- <sup>19</sup> DALE, W. M., GRAY, L. H. and MEREDITH, W. J., *Phil. Trans.*, 1949, **242A**, 33
- <sup>20</sup> GRAY, L. H., *Brit. J. Radiol.*, 1953, **26**, 609
- <sup>21</sup> LEA, D. E., *Actions of Radiations on Living Cells*, Cambridge, 1946
- <sup>22</sup> FRICKE, H. and HART, E. J., *J. chem. Phys.*, 1935, **3**, 60
- <sup>23</sup> FRICKE, H. and HART, E. J., *ibid.*, 1938, **6**, 229
- <sup>24</sup> ALEXANDER, P., unpublished
- <sup>25</sup> DAINTON, F. S. and ROWBOTTOM, J., *Trans. Faraday Soc.*, 1953, **49**, 1160
- <sup>26</sup> ALLEN, O. A., *Disc. Faraday Soc.*, 1952, **12**, 79
- <sup>27</sup> NIIRA, K., *J. phys. Soc. Japan*, 1952, **7**, 191
- <sup>28</sup> BURTON, M., MAGEE, J. L. and SAMUEL, A. M., *J. chem. Phys.*, 1952, **20**, 760
- <sup>29</sup> JAFFE, G., *Ann. Phys., Lpz.*, 1913, **62**, 303
- <sup>30</sup> SAMUEL, A. H. and MAGEE, J. L., *J. chem. Phys.*, 1953, **21**, 1080
- <sup>31</sup> PLATZMAN, R. L. and FROELICH, H., *Phys. Rev.*, 1953, **92**, 1152
- <sup>32</sup> ALLEN, O. A., *J. phys. Colloid Chem.*, 1948, **52**, 579
- <sup>33</sup> FRICKE, H. and HART, E. J., *J. chem. Phys.*, 1935, **3**, 596
- <sup>34</sup> BONET-MAURY, P., *Disc. Faraday Soc.*, 1952, **12**, 72
- <sup>35</sup> RIGG, T., *ibid.*, 1952, **12**, 119
- <sup>36</sup> DAINTON, F. S. and SUTTON, H. C., *Trans. Faraday Soc.*, 1953, **49**, 1011
- <sup>37</sup> HART, E. J., *J. phys. Chem.*, 1952, **56**, 594
- <sup>38</sup> HOCHANADEL, C. J., *ibid.*, 1952, **56**, 587
- <sup>39</sup> FRICKE, H., *J. chem. Phys.*, 1935, **3**, 364
- <sup>40</sup> ALDER, M. G. and EYRING, H., *Nucleonics*, 1952, **10** (No. 4), 54
- <sup>41</sup> STEIN, G. and WEISS, J., *J. chem. Soc.*, 1949, p. 3256
- <sup>42</sup> SWORSKI, T. J., *J. chem. Phys.*, 1952, **20**, 1817  
WRIGHT, J., *Disc. Faraday Soc.*, 1952, **12**, 114
- <sup>43</sup> COLLINSON, E. and DAINTON, F. S., *ibid.*, 1952, **12**, 212
- <sup>44</sup> ALPER, T., EBERT, M., GRAY, L. H., LEFORT, M., SUTTON, H. C. and DAINTON, F. S., *ibid.*, 1952, **12**, 266
- <sup>45</sup> BARB, W. G., *ibid.*, 1952, **12**, 273  
BAMFORD, C. H. and JENKINS, A. D., *Proc. Roy. Soc.*, 1953, **216A**, 515
- <sup>46</sup> GOLDBLITH, S. A. and PROCTOR, B. E., *Nucleonics*, 1949, **5** (No. 2), 50
- <sup>47</sup> LEFORT, M., *J. chim. phys.*, 1950, **47**, 784
- <sup>48</sup> GOLDBLITH, S. A. and PROCTOR, B. E., 1948, **3** (No. 2), 32
- <sup>49</sup> GRAY, L. H., CONGER, A. D., EBERT, M., HORNSEY, S. and SCOTT, O. C. A., *Brit. J. Radiol.*, 1953, **26**, 638
- <sup>50</sup> ALEXANDER, P. and FOX, M., *Trans. Faraday Soc.*, 1954, **50**, 605
- <sup>51</sup> LEFORT, M., *C.R. Acad. Sci., Paris*, 1953, **237**, 159
- <sup>52</sup> KOLTHOFF, I. M. and MEDALIA, A. I., *J. Amer. chem. Soc.*, 1949, **71**, 3789
- <sup>53</sup> WATERS, W. A., *J. chem. Soc.*, 1946, 1153; *Disc. Faraday Soc.*, 1947, **2**, 179
- <sup>54</sup> FRICKE, H. and BROWNSCOMBE, E. R., *J. Amer. chem. Soc.*, 1933, **55**, 2358
- <sup>55</sup> HAISINSKY, M., *Disc. Faraday Soc.*, 1952, **12**, 133
- <sup>56</sup> DAINTON, F. S., *J. chem. Soc.*, 1952, p. 1533
- <sup>57</sup> DAINTON, F. S. and COLLINSON E., *Annu. Rev. phys. Chem.*, 1951, **2**, 99

THE RADIATION CHEMISTRY OF AQUEOUS SYSTEMS

- <sup>58</sup> WEISS, J., *Nature*, 1950, **165**, 728
- <sup>59</sup> RIGG, T., STEIN, G. and WEISS, J., *Proc. Roy. Soc.*, 1952, **211A**, 375
- <sup>60</sup> DEWHURST, H. A., *Trans. Faraday Soc.*, 1953, **49**, 1174
- <sup>61</sup> ABEL, E., *Z. physik. Chem.*, 1953, **201**, 108
- <sup>62</sup> JOHNSON, E. R., *U.S. Rep. BNL* 1352
- <sup>63</sup> COULSON, C. A., *Proc. Roy. Soc.*, 1952, **122A**, 385
- <sup>64</sup> HAISINSKY, M. and MAGAT, M., *C.R. Acad. Sci., Paris*, 1951, **233**, 954
- <sup>65</sup> BARTINDALE, G. W. R., *Disc. Faraday Soc.*, 1952, **12**, 244  
DAINTON, F. S., *ibid.*, 1952, **12**, 212
- <sup>66</sup> URI, N., *Chem. Rev.*, 1952, **50**, 375
- <sup>67</sup> BURTON, M., *Symposium on Radiobiology*, Wiley, New York, 1950, p. 117
- <sup>68</sup> ALEXANDER, P. and FOX, M., *Nature*, 1952, **170**, 1022
- <sup>69</sup> FRICKE, H., *J. chem. Phys.*, 1934, **2**, 556
- <sup>70</sup> EBERT, M. and BOAG, J. W., *Disc. Faraday Soc.*, 1903, **12**, 189
- <sup>71</sup> FORSSBERG, A. G., *Acta radiol. Suppl.* 49, 1943
- <sup>72</sup> HART, E. J. and MATHESON, M. S., *ibid.*, 1903, **12**, 169
- <sup>73</sup> ALEXANDER, P. and BACQ, Z. M. et al., *Radiation Res.*, 1954, **1** (in the Press)
- <sup>74</sup> KAILAN, A., *S. B. Akad. Wiss. Wien*, 1921, **130** (11a), 307, 469
- <sup>75</sup> KAILAN, A., *ibid.*, 1922, **131** (11a), 569; 1924, **133** (11a), 477
- <sup>76</sup> HART, E. J., *J. Amer. chem. Soc.*, 1951, **73**, 68
- <sup>77</sup> DALE, W. M., DAVIES, J. V. and GILBERT, C. W., *Biochem. J.*, 1949, **45**, 93
- <sup>78</sup> BHATIA, D. S. and PROCTOR, B. E., *ibid.*, 1951, **49**, 550
- <sup>79</sup> DALE, W. M., DAVIES, J. V. and GILBERT, C. W., *ibid.*, 1949, **45**, 543
- <sup>80</sup> BARRON, E. S. G., *Symposium on Radiobiology*, Wiley, New York, 1950, p. 216
- <sup>81</sup> DALE, W. M. and DAVIES, J. V., *Nature*, 1949, **163**, 64
- <sup>82</sup> DALE, W. M., *Biochem. J.*, 1951, **48**, 129
- <sup>83</sup> MAR, P. G. and TSCHAPEROFF, I. G. C., *Science*, 1951, **113**, 549  
CORSON, M., *Arch. Biochem. Biophys.*, 1951, **33**, 263
- <sup>84</sup> WIITCIER, S. L., *Naturwissenschaften*, 1952, **39**, 450
- <sup>85</sup> ERRERA, J. and HENRI, V., *J. Phys. Radium*, 1926, **7**, 225
- <sup>86</sup> BOYLAND, E. and SIMS, P., *J. chem. Soc.*, 1953, p. 2966
- <sup>87</sup> MILLER, N., *Nature*, 1953, **171**, 688
- <sup>88</sup> HUBER, W., *Naturwissenschaften*, 1951, **38**, 21
- <sup>89</sup> DITTRICH, W. and SCHUBERT, G., *Strahlentherapie*, 1953, **92**, 532
- <sup>90</sup> FRITZ-NIGGLI, H., *Schweiz. med. Wschr.*, 1951, **81**, 1218
- <sup>91</sup> CROWTHER, J. A. and FAIRBROTHER, L. *Phil. Mag.*, 1927, **4**, 325
- <sup>92</sup> CROWTHER, J. A., LIEBMANN, M. and LANE, T. B., *ibid.*, 1937, **24**, 654
- <sup>93</sup> LOISLEUR, J. and LATARJET, R., *Bull. Soc. Chim. biol.*, 1942, **24**, 172
- <sup>94</sup> SWORSKI, T. J., *Radiation Res.*, 1954, **1**, 231
- <sup>95</sup> BARRON, E. S. G. and JOHNSON, P., *Arch. Biochem.*, 1952, **41**, 188
- <sup>96</sup> Compare conflicting reports by FIQUET, F. and BERNAS, A., *J. Chim. phys.*, 1954, **51**, 47 and DAINTON, F. S. *Brit. Emp. Cane. Campgn. Rep.* 1954, **31**, 202
- <sup>97</sup> GRAY, H. L., READ, J. and LIEBMANN, M., *Brit. J. Radiol.* 1941, **14**, 102
- <sup>98</sup> KINSEY, V. E., *J. biol. Chem.*, 1935, **110**, 551

## EFFECT OF RADIATION ON MACROMOLECULES

ONE of the most characteristic features of radiation biology is the extremely small amount of energy required to bring about major changes. The equal accessibility of all parts of the organism and the production of high local concentration of reactive chemicals (*e.g.* molar H<sub>2</sub>O<sub>2</sub> in the track of an  $\alpha$ -particle) contribute to the high efficiency. Reaction with small molecules such as vitamins, essential growth factors, sterols, ATP or sulphydryl compounds like glutathione could not bring about the biological changes observed as only a small fraction of the available material could possibly be affected.

The primary process of radiation is therefore likely to be reaction with vital macromolecules where a change involving one atom can result in the inactivation of a structure containing hundreds of thousands of atoms. The available information concerning the effect on macromolecules of ionizing radiation both directly and *via* free radicals is of immediate interest for the understanding of radio-biological processes. The structure of few naturally occurring macromolecules has been fully established and for some, such as the nucleic acids, not even the most fundamental properties such as molecular weight, are known with certainty (see p. 144). For this reason synthetic polymers of known size and constitution have proved more suitable for determining the type of change which can be brought about by ionizing radiation.

### DIRECT ACTION ON SYNTHETIC MACROMOLECULES

*Crosslinking\* and degradation*—From general considerations discussed in Chapter 1 it is clear that the number of reactions which can occur when an organic macromolecule (liquid or solid) is irradiated are limited. Due to the 'cage' effect, dissociation into large radicals is unlikely as immediate recombination would occur. The two most probable reactions are (i) the loss of a hydrogen atom (and possibly

---

\* When one macromolecule is linked to another this chemical reaction is referred to as crosslinking. If one part of a macromolecule is chemically linked to another part of the same molecule this is referred to as internal or intra-molecular crosslinking.

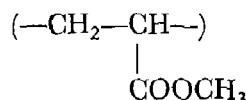
### EFFECT OF RADIATION ON MACROMOLECULES

a methyl radical) as this can diffuse out of the 'cage' leaving a radical macromolecule, and (ii) the dissociation into two stable molecules which cannot recombine. The extensive investigations carried out in the last few years on the effect of  $\gamma$ -rays as well as of pile irradiation (consisting of a mixture of neutron and  $\gamma$ -rays) on a large number of synthetic macromolecules can be satisfactorily interpreted on these premises<sup>1</sup>.

The major effects are either crosslinking to give a polymer which is no longer soluble in suitable solvents, or main-chain degradation leading to a decrease in molecular weight. The extent of the cross-linking can be determined from the degree of swelling of the polymer in solvents in which it was soluble before irradiation. From the change in molecular weight—usually determined from viscosity measurements—the number of main-chain breaks produced can be calculated. The energy necessary to produce a crosslink or break varies widely from one polymer to another. The available data is summarized in *Table I*, all the irradiations were carried out either in the pile or a cobalt unit giving pure  $\gamma$ -rays. Despite the presence of particles having a high specific ionization in the pile irradiation, strictly comparable results were obtained with  $\gamma$ -rays from a cobalt unit.

In conformity with this theory, the gas released on irradiation in the highest yield is hydrogen. The methacrylates in addition to hydrogen liberate large quantities of CO and CO<sub>2</sub> which arise from the dissociation of the ester group. This can rearrange as shown in *Table I* to give off a stable molecule which cannot be trapped by the cage effect. This reaction is completely analogous to the decomposition by deuterons of long-chain fatty acids (see p. 41). For polymethylmethacrylate it would appear that approximately equal numbers of main chains and side chains were broken. The gases given off during the irradiation are trapped within the polymer at high pressures. On heating, the polymer becomes plastic and the gases can coalesce to form large bubbles and a foam-like structure as shown in *Figure 1*.

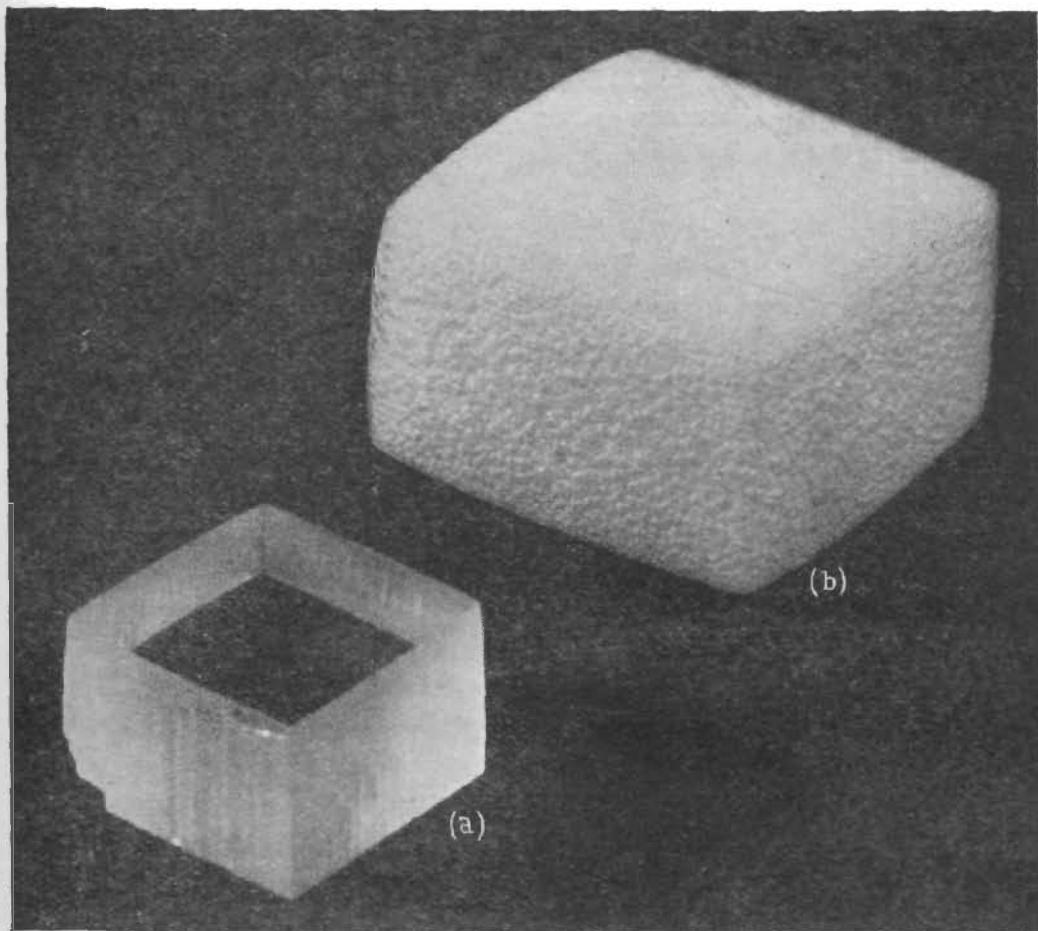
The polyacrylates (*e.g.* polymethyl acrylate)



are an interesting case since the side chains can decompose, but a rearrangement along the main chain is not possible. On irradiation therefore the main chains become crosslinked to give an in-

soluble product while release of gases due to side chain breakdown takes place in the same way as in the methacrylates.

In the crosslinking reactions a minimum dose has to be given before any part of the sample becomes insoluble or infusible\*; once this value known as the gel point has been exceeded the proportion of insoluble material increases rapidly till at twice this dose 80 per



*Figure 1. On irradiating polymethylmethacrylate, gases are evolved due to the decomposition of the side chain. When the polymer is warmed the gases coalesce and produce an expanded and 'bubbled' polymer<sup>1</sup>. (a) Polymer immediately after irradiation, (b) Irradiated polymer after heating to 100° C*

cent of the material is insoluble<sup>2</sup>. To render a polymer insoluble it is necessary to form a crosslinked network of infinite size. The

\* The melting point of a polymer is largely independent of molecular weight. As crosslinking proceeds the melting point hardly changes until a point is reached where a small fraction is 'infinitely' crosslinked when the melting point jumps up sharply to a value where thermal decomposition occurs. At this point the polymer is termed infusible. In fact only a small fraction is infusible, but this provides a honeycomb-like network which prevents the molten material from escaping.

## EFFECT OF RADIATION ON MACROMOLECULES

 Table I. Effect of  $\beta$ -rays and  $\gamma$ -rays on Solid Polymers<sup>1-8</sup>

Compound irradiated	Predominant reaction	Possible mechanism	Energy required per main-chain break or per crosslink
<i>Polyethylene (and other straight-chain paraffins)</i>	crosslinking	$-\text{CH}_2-\text{CH}_2 \rightarrow -\text{CH}_2-\dot{\text{C}}\text{H}- + \text{H}$ <i>(polymer radicals combine to form macro-network)</i>	20 eV (approx.)
Rubber	crosslinking	$2\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_2 \rightarrow -\text{CH}_2-\text{CH}=\text{CH}-\dot{\text{C}}\text{H}- + \text{H}$	
Polystyrene	crosslinking		
Polyisobutylene	degradation	$\begin{array}{c} \text{CH}_3 & \text{CH}_3 \\   &   \\ -\text{CH}_2-\text{C}-\text{CH}_2-\text{C}- & \rightarrow -\text{CH}_2-\text{C}=\text{CH}_2 + \text{CH}_3-\text{C}- \\   &   \\ \text{CH}_3 & \text{CH}_3 \end{array}$ <i>(internal rearrangement to produce two stable molecules)</i>	17 eV

## DIRECT ACTION ON SYNTHETIC MACROMOLECULES

Table I.—continued

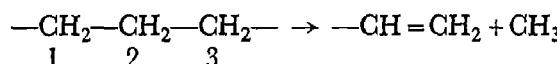
Compound irradiated	Predominant reaction	Possible mechanism	Energy required for main-chain break or per crosslink
<i>Poly(methylmethacrylate</i>	<i>degradation</i>	<p>(1) <i>main chain</i></p> $\begin{array}{c} \text{CH}_3 \\   \\ -\text{CH}_2-\text{C}-\text{CH}_2-\text{C}- \\   \quad   \\ \text{COOCH}_3 \quad \text{COOCH}_3 \end{array} \rightarrow \begin{array}{c} \text{CH}_2 \\    \\ -\text{CH}_2-\text{C}-\text{COOCH}_3 \end{array} + \begin{array}{c} \text{CH}_3 \\   \\ \text{COOCH}_3 \end{array}$ <p>(2) <i>side chain</i></p> $\begin{array}{c} \text{CH}_3 \\   \\ -\text{CH}_2-\text{C}-\text{CH}_2-\text{CO}_2 \\   \\ \text{CH}_3 \end{array} \rightarrow \begin{array}{c} \text{CH}_3 \\   \\ \text{CH}_2-\text{C}-\text{CH}_2-\text{CO}_2 \\   \\ \text{CH}_3 \end{array}$ <p>or</p> $\begin{array}{c} \text{CH}_3 \\   \\ -\text{CH}_2-\text{C}-\text{CH}_2-\text{CO} \\   \\ \text{O} \\   \\ \text{CH}_3 \end{array}$	61 eV per main-chain break + associated side-chain breaks
<i>Polyvinyl alcohol</i>		<p><i>crosslinking and degradation, the former predominates</i></p> $\begin{array}{c} \text{CH}_3 \\   \\ -\text{CH}_2-\text{C}-\text{CH}_2-\text{CH}- \\   \quad   \\ \text{OH} \quad \text{OH} \end{array} \rightarrow \begin{array}{c} \text{OH} \\   \\ -\text{CH}-\dot{\text{C}}\text{H}- + \text{H} \end{array} \quad \begin{array}{l} \text{followed by} \\ \text{crosslinking} \\ (\text{degrades}) \end{array}$ $\begin{array}{c} \text{CH}_2-\text{CH}-\dot{\text{C}}\text{H}-\text{CH}_2+\text{CH}_3-\text{CH}- \\   \quad   \\ \text{OH} \quad \text{OH} \end{array}$ <p><i>degradation</i></p> $-\text{CF}_2-\text{CF}_2 \rightarrow \text{fluorine evolved which degrades polymer}$	

### EFFECT OF RADIATION ON MACROMOLECULES

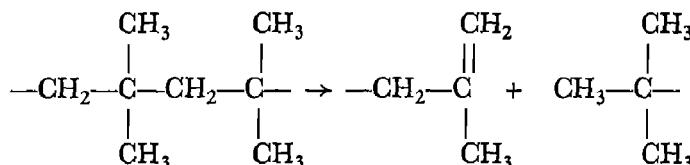
gel point coincides with an amount of reaction when statistically one crosslink has been formed for each molecule present on a weight average basis for polydisperse materials. The energy necessary to form one crosslink is approximately independent of the molecular weight of the polymer<sup>9</sup>. This means that the dose necessary to reach gel point varied inversely as the molecular weight of the initial material.

Straight paraffins do not undergo main-chain degradation on irradiation presumably because no facile rearrangement is possible to give two stable molecules.

For example, the reaction:



can only occur when the molecule is in an improbable configuration where C<sup>1</sup> is in close proximity to C<sup>3</sup>. For this reason crosslinking is the predominant reaction for straight-chain hydrocarbons. For substituted hydrocarbons such as polyisobutylene<sup>5</sup> there are no steric restrictions on the dissociation



for which there is ample energy available.

The great variation in the energy necessary to produce one cross-link (or to break one main-chain bond) in different materials is important since it disproves the widely held hypothesis (see p. 61) that once an atom is ionized the molecule of which it is a part undergoes a chemical change. The corollary that only one primary chemical reaction occurs for each ionization, *i.e.* for every 32.5 eV of x- or  $\gamma$ -rays energy taken up, is also not supported. In polystyrene obviously a large part of the energy absorbed is dissipated in the aromatic benzene ring without reaction. The radiation resistance of aromatic structures probably arises from the fact that the energy is distributed over the whole molecule extremely rapidly. A particularly interesting case is polyisobutylene which requires only 17 eV per main-chain break. This means that for every ionization (if this is assumed by analogy to require 32.5 eV, see p. 23) two distinct reactions take place. These cannot occur on neighbouring groups—when the same ‘packet’ of energy could be responsible—as the experimental method used would only count this as one break.

#### DIRECT ACTION ON SYNTHETIC MACROMOLECULES

The degradation of polyisobutylene is perhaps the first clear example of a radiochemical reaction in a condensed system where excitations contribute to the overall yield. As in these polymer systems there is no correlation between yield and the number of ionizations produced it would seem to be preferable to treat the energy uptake as one process and not to separate it into excitations and ionizations since there may be no sharp division between the two.

The possibility that both crosslinking and degradation reactions may occur in biological systems cannot be excluded, and the complexity of the biological response may in part be due to the fact that different macromolecules are altered quite differently by radiations.

*Energy transfer*—In gases (see p. 38) and in aqueous solutions (see p. 47) there is abundant evidence that the molecule which absorbs the energy need not be the one which undergoes the observed chemical reactions. This exchange takes place *via* free radicals in aqueous solutions and by charge and energy transfer in gases. Recently MANION and BURTON<sup>10</sup> found that the decomposition of cyclohexane was decreased in the presence of benzene which took up the energy from the cyclohexane molecules before these had dissociated (see p. 42).

In all these cases the molecules concerned were in frequent collision and the first evidence that energy from ionizing radiations can be transferred within solids, when the molecules are relatively immobile, was obtained from experiments on the irradiation of polymethylmethacrylate. When films of this material containing added substances were prepared, the degradation was less and the extent of the reduction was found to depend on the nature and quantity of the additive<sup>1, 11</sup> (see *Table II*). As the amount of energy taken up by the polymer is not changed by the presence of another substance it follows that energy has been transferred to the other substance. In support of this view it is found that a greater proportion of the additive is decomposed by a given dose of radiation when incorporated in the polymer than when irradiated by itself<sup>11</sup>. The additive can be considered as a protective agent or alternatively the polymer can be considered as an energy reservoir. As much as 75 per cent of the energy supplied to the whole system can be taken up by 10 per cent of an additive, *i.e.* four times the dose necessary to break one main-chain bond of the polymer and the energy taken up by the additive is seven times that originally received. The amount of protection given this way is (i) independent of the dose until this is sufficiently large to decompose an appreciable fraction of the additive, and (ii) proportional to the concentration of the

## EFFECT OF RADIATION ON MACROMOLECULES

*Table II. Protective Action of Different Substances when incorporated in Solid Polymethylmethacrylate which is being degraded by Ionizing Radiations*

Substance (10% by weight)	% Protection $\left(\frac{R- Re}{R}\right)^*$	Substance (10% by weight)	% Protection $\left(\frac{R- Re}{R}\right)^*$	Substance (10% by weight)	% Protection $\left(\frac{R- Re}{R}\right)^*$
Di-m-tolyl thiourea	72.0	$\beta$ -Naphthol	55.7	Phenanthrene	47.9
Amine	64.2	Pyrene	54.0	Allyl glycidyl ether	37.3
Phenol	64.2	$\beta$ -Mercapto- ethylamine	53.7	Di-methyl urea	33.6
$\alpha$ -Naphthyl- amine	60.6	$\beta$ -Naphthyl- amine	51.5	Nonanol	29.1
Allyl thiourea	57.0	$\alpha$ -Naphthol	50.7	Nonylic acid	23.0
8-Hydroxy- quinoline	55.7	Naphthalene	49.6	Ethyl urea	17.5
				Medicinal paraffin	0.0

\* R is the dose given and Re is the dose required to produce the same amount of degradation in the absence of the protective agent.

additive. Since the fraction of the polymer molecules in physical contact with the protective agent can only be 10 per cent it follows that the large amount of energy transfer is only possible if the energy—initially absorbed at one or two points—is distributed over large areas. The fact that protection is possible provides direct support for the mechanism of degradation involving a rearrangement which has to wait (*e.g.*  $10^{-6}$  sec) for a suitable steric disposition of the molecule.

Transfer of energy absorbed in one part of a macromolecule to another\* was demonstrated by SVEDBERG and BROHULT<sup>12</sup> for the giant protein haemocyanin (see p. 135). Similar processes have also been found in synthetic macromolecules by ALEXANDER and CHARLESBY<sup>11</sup>. Thus a copolymer of isobutylene with 20 per cent styrene is much more resistant to degradation than polyisobutylene, the energy to break a main-chain bond being increased from 17 to 32 eV. The protection in this case arises from the fact that energy absorbed in the isobutylene part of the chain is transferred to the styrene part where it is dissipated in the aromatic ring system with only a very small amount of chemical change taking place. In a

\* The normal processes of internal conversion discussed on p. 33 provides for the equipartition of energy throughout a molecule, so that in a large molecule insufficient energy for a chemical change will be centred in any one bond; a different mechanism is necessary for the localization of energy in one area when the absorption has occurred in another.

## DIRECT ACTION ON SYNTHETIC MACROMOLECULES

similar way the energy required to form a crosslink in a straight-chain hydrocarbon (measured as the dose to render the material infusible) was greatly increased by the presence of an aromatic ring. The addition of a saturated polycyclic hydrocarbon decalin at the centre of the dodecane chain slightly increases the dose required to produce a crosslink, whereas the presence of the aromatic naphthalene ring may render the straight chain much more difficult to crosslink, although any steric factors which would influence the reaction will be very similar in the two cases.

As the amount of energy absorbed by the paraffin chain is not altered by the addition of other groups, it must be concluded that the energy has been transferred to the aromatic ring before it could bring about the chemical changes leading to crosslinking. *Table III* shows that maximum protection occurs when the aromatic ring is at the centre of the paraffin chain and that the effect is smallest at

*Table III.* Dose (in Pile Units\*) required to render Substituted Dodecanes Infusible

Substituent	1-dodecane	4-dodecane	6-dodecane
1' naphthyl .	43	—	—
2' naphthyl .	43	62	66
2' decalyl .	—	—	37

\* The pile unit is defined as a flux of  $10^{17}$  slow neutrons/cm<sup>2</sup> plus the associated  $\gamma$ -rays and fast neutrons. Comparison of crosslinking and degradation produced in the pile and by  $\gamma$ -rays from a cobalt unit indicate that in these polymer reactions one pile unit is equivalent to  $4.5 \times 10^7$  r of  $\gamma$ -rays. Unsubstituted dodecane requires 27 units to be rendered infusible.

the end. This suggests that energy transfer can occur along a saturated chain of 12 carbon atoms but that it is more effective over shorter distances.

The occurrence of energy transfer processes in organic molecules may play an important part in the biological actions of ionizing radiations (compare p. 62), since, as we have shown here, such transfer can result both in a decrease of total reaction by concentrating the energy in a radiation resistant group, or in the preferential decomposition of a particular component. For example, the possibility can now be envisaged of protecting against a 'direct effect' since macromolecules need not decompose immediately on becoming ionized and can transfer their energy without undergoing reaction if a suitable receptor is present. Consequently it cannot be concluded that any effect which can be protected against by the addition of chemicals must invariably be the result of reactions with free radicals produced in the surrounding medium.

#### EFFECT OF RADIATION ON MACROMOLECULES

For the smaller viruses, LEA<sup>13</sup> had found (see p. 58) that an ionization anywhere within the organism leads to inactivation. The conclusion that every part of the virus was equally vulnerable is in contradiction to biochemical experiments which had shown that many groups of the macromolecule could be modified without loss of activity. The results reported here indicate that the difficulty may be resolved if the target represents the distance over which energy transfer can take place while the sensitive region is smaller. If this view is accepted, the 'direct' and 'indirect' actions are not fundamentally different but represent merely two different mechanisms by which energy provided by ionizing radiations to the system as a whole is concentrated at definite points. The reactions produced in a complex structure by direct energy transfer may be more selective than those brought about by the indirect action of highly reactive free radicals and this may explain the much higher efficiency of direct action for the inactivation of viruses (see p. 51).

#### INDIRECT ACTION ON SYNTHETIC MACROMOLECULES

So far the effect of ionizing radiations on aqueous solutions of macromolecules has been examined with three synthetic materials, all of which react quite differently. Polyvinyl alcohol<sup>6</sup> is crosslinked, polymethacrylic acid is degraded<sup>14</sup> and polymetaphosphate<sup>15</sup> appears to be unaffected.

*Crosslinking*—The crosslinking of polyvinyl alcohol solutions by  $\gamma$ -rays follows a course very similar to that of pure polymers. At first there is very little change in the solution as an increase in molecular weight produced by crosslinking is not reflected in a great increase in viscosity\*. At a well-defined dose the solution sets to a gel when presumably enough crosslinks have been produced to make part of the material into an infinite network. With greater amount of radiation the number of crosslinks increases and the gel becomes less swollen until it no longer occupies the same volume as the original solution and the irradiated solution consists of a central core of gel surrounded by water. As the dose is increased the gel continues to contract until, depending on the initial concentration, maximum degree of crosslinking is obtained which is not increased by further irradiation.

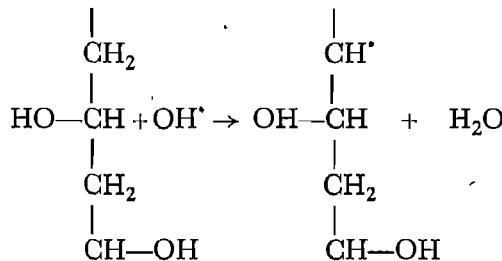
The most probable reaction is abstraction of a hydrogen atom by

---

\* The viscosity of polymer solutions is a function of the chain length of the polymer; crosslinking produces branched chains, the viscosity of which is not much greater than that of the longest chain.

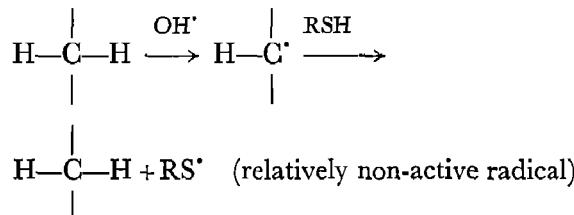
### INDIRECT ACTION ON SYNTHETIC MACROMOLECULES

an OH radical formed by the ionization of water to give a polymer radical:



which can combine with another polymer radical to give a cross-linked structure. In addition to these recombinations the polymer radical must be deactivated in alternative processes since a gel is only formed if the initial concentration of polyvinyl alcohol exceeds 0.3 per cent. As the concentration is increased the dose necessary to produce a gel is greater, but beyond this point the crosslinking proceeds more effectively; with a 0.5 per cent solution the gel crosslinked to its maximum is capable of taking up 25 times its weight of water while for a 10 per cent gel the corresponding value is 9 times.

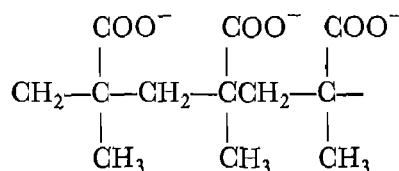
No crosslinking occurs in the presence of dissolved oxygen which probably reacts selectively with the polymer radical which cannot recombine to give stable bonds. Crosslinking can also be prevented by the addition of relatively small quantities of sulphhydryl compounds (*e.g.*  $\beta$ -mercapto ethylamine) which act as transfer agents (Chapter 14) by donating a hydrogen atom to the polymer radical and thereby rendering it non-reactive:



*Degradation*—On irradiation with  $\alpha$ - or  $\gamma$ -rays the viscosity of aqueous solutions of polymethacrylic acid is decreased<sup>14</sup> and direct molecular weight determinations showed that this was due to breaks produced in the main chain. Crosslinking is never observed and degradation was found in solutions of concentration ranging from 0.01 per cent to pure polymer. The efficiency of degradation is independent of concentration since the energy required to break a main-chain bond is approximately the same whether the reaction

### EFFECT OF RADIATION ON MACROMOLECULES

is produced directly by irradiating the dry polymer or indirectly by irradiating the solutions. This is a coincidence since the mechanism is of course quite different, the direct action being closely similar to that of polymethylmethacrylate as described on p. 125, while the indirect action is brought about by a specific radical. The change in viscosity can be used to detect very small doses of radiation if a high molecular weight (*e.g.*  $2 \times 10^6$ ) polymer is used. When dissolved as its salt (usually the sodium salt) the polymer is extremely viscous because the carboxyl groups along the chain repel one another and enforce an extremely asymmetric shape on the molecule:



the neutralizing cations (*e.g.* sodium) are dissociated and are distributed throughout the solution without preventing the repulsion along the chain\*. Thus a 0.025 per cent solution of sodium methyl methacrylate having a molecular weight (number average)<sup>†</sup> of  $10^6$  and a high viscosity is  $2.5 \times 10^{-7}$  m with respect to polymer molecules. If one break results from each ion pair produced (*i.e.* each H or OH radical) then a dose of 100 r should lower the molecular weight by half. The observed efficiency is about half this and the 'G' value for main-chain bonds broken is about 1.6. *Figure 2* shows how in this extremely sensitive system 50 r can readily be detected.

This system is of some biological interest since the degradation only occurs in the presence of oxygen; *Figure 8* on p. 106 shows the influence of the concentration of oxygen on the degradation<sup>17</sup>. Clearly the primary radical formed in water (*i.e.* OH and H(?)) does not degrade the polymer. The presence of oxygen increases the yield of hydrogen peroxide, but the polymer is quite stable towards this reagent. The most probable agent for the degradation is therefore the HO<sub>2</sub> radical which is produced in high yield by x- and

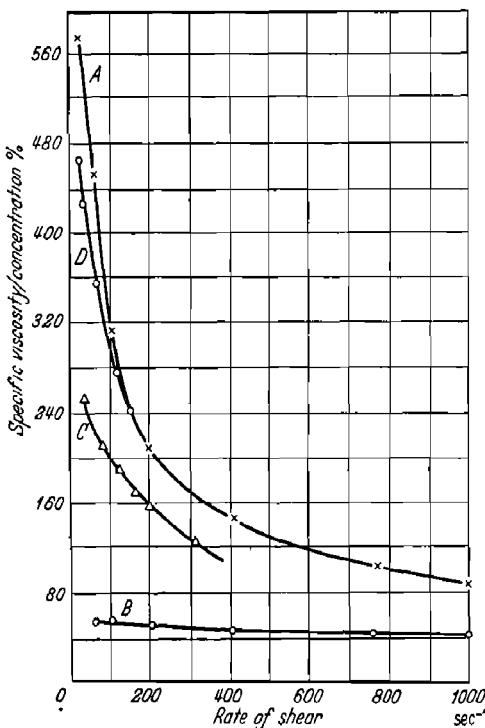
\* These solutions do not behave as ordinary liquids and their viscosity varies with the speed (the rate of shear) with which it is forced through the capillary (see *Figure 2*). This behaviour is referred to as non-Newtonian and necessitates the use of a special viscometer in which the shear rate can be determined<sup>16</sup>.

† There are several different ways of expressing the average molecular weight of a polymer containing molecules differing in molecular weight. Viscosity measurements which favour large molecules, give a so-called weight average ( $\bar{M}_w$ ) while a number average ( $\bar{M}_n$ ) is required for determining the number of breaks produced by radiation. Approximately  $\bar{M}_w = 2\bar{M}_n$  for the type of polymer used<sup>1</sup>.

### INDIRECT ACTION ON SYNTHETIC MACROMOLECULES

$\gamma$ -rays in aerated water (see p. 104). The alternative view, that the degradation is the result of successive reactions involving first attack by an OH radical followed by addition of oxygen to the radical formed has been excluded by experiments<sup>15, 17</sup> which need not be detailed here. The failure of OH radicals to lower the viscosity of polymethacrylic acid does not necessarily imply that these radicals do not react with the polymer but only that they do not react in a way to bring about a break in the main chain. The degradation of PMA which resembles closely in a number of respects

*Figure 2. Effect of 400-kV x-rays on viscosity of 60 per cent neutralized high molecular weight polymethacrylic acid<sup>14</sup>: A, 0.08 per cent polymethacrylic acid; B, 0.08 per cent polymethacrylic acid subjected to 4000 r; C, 0.025 per cent polymethacrylic acid subjected to 200 r; D, 0.025 per cent polymethacrylic acid subjected to 50 r*



certain biological effects produced by x-rays (Chapter 14) focusses attention on the role of  $\text{HO}_2$  radicals in bringing about radiobiological effects. In particular the  $\text{HO}_2$  radical may play an important part in the enhancing effect of oxygen on x- and  $\gamma$ -rays which is so widely observed (see Chapter 8).

Some polymers are extremely resistant to the free radicals produced in water. Solutions of a high molecular weight polymetaphosphate are unaffected by radiation doses (see *Figure 3*) which would reduce the viscosity of comparable solutions of polymethacrylic acid to one-tenth. The P—O linkage is singularly resistant

### EFFECT OF RADIATION ON MACROMOLECULES

therefore to OH, HO<sub>2</sub> and H(?) radicals as well as to hydrogen peroxide in spite of the fact that it is slowly hydrolysed by water and

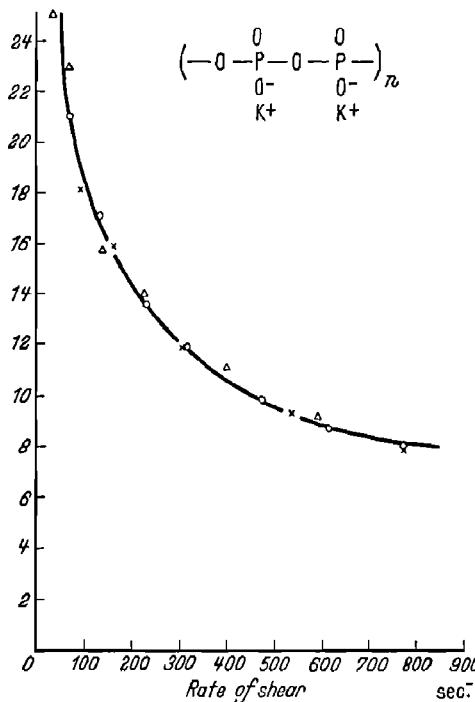


Figure 3. Viscosity of a 0.1 per cent soln of potassium polyphosphate of molecular weight  $2 \times 10^6$ . This polymer is not degraded by irradiation by x-rays either in the presence or in the absence of oxygen. ○ Polymer alone; × Irradiated with  $10^4$  r of x-rays in the presence of air; △ Similarly irradiated in the absence of air 15

from a general chemical point of view would not be considered exceptionally stable.

### CHANGES PRODUCED IN PROTEINS

*Direct action*—A considerable number of proteins having enzymatic and other biological activity have been irradiated in the dry state or in highly concentrated solutions where direct action predominates. As shown in Chapter 2 the single-hit target theory can be applied to almost all the data and gives target sizes in good agreement with the physical dimensions of the molecules. This work throws no light on the chemical changes brought about by the ionizing radiations. Energy transfer processes, similar to those discussed on p. 127, can take place in some proteins (see below) and the observation that one ionization per molecule results in inactivation need not therefore imply that every part of the protein molecule is equally vulnerable.

One of the earliest reports of the effect of x-rays on dry proteins was made by ASTBURY and Woods<sup>18</sup> who found that the physical

## CHANGES PRODUCED IN PROTEINS

properties of wool fibres were greatly modified by ionizing radiations. The most readily detectable effects were an increase in the amount of supercontraction and a decreased tendency to acquire a permanent set. The observed changes in these complex phenomena can be brought about either by breaking peptide bonds in the main chain or by severing disulphide bonds which link the chains<sup>19</sup>. Preliminary experiments indicate that the disulphide bonds are very resistant to radiation and that the peptide bonds are attacked. In support of this view it is found that silk fibres, which do not have disulphide crosslinks, are weakened much more readily than wool fibres which show considerable resistance to radiation<sup>20</sup>.

The giant protein molecules, the haemocyanins, are found in the blood of certain arthropods and molluscs and were shown by Svedberg to have molecular weights of up to ten million. The

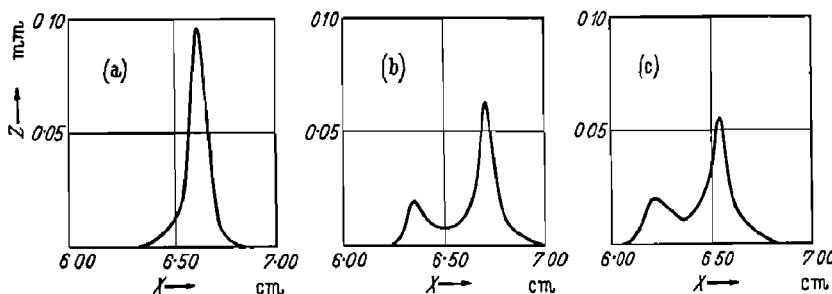


Figure 4. Splitting of haemocyanin molecule by the direct action of  $\alpha$ -particles. (a) Ultracentrifuge sedimentation diagram of haemocyanin before irradiation; (b) Ultracentrifuge sedimentation diagram of haemocyanin after irradiation at  $20^\circ\text{C}$ ; (c) Ultracentrifuge after irradiation at  $-180^\circ\text{C}$ . The original peak corresponds to a material of molecular weight of  $9 \times 10^6$  while the component produced by irradiation, clearly a homogeneous product, has a molecular weight of  $4.5 \times 10^6$

haemocyanin from the snail *Helix pomatia* has received the most detailed study and is shown to be made up of identical sub-units into which it can be reversibly dissociated by changes in pH away from the isoelectric point, increase in ionic concentration, and by the addition of hydrogen-bond breaking reagents such as urea<sup>21</sup>. The molecules having an initial molecular weight of  $8.9 \times 10^6$  first dissociate into halves and finally into eighths. These fragments are held together by secondary valency forces such as hydrogen bonds and salt links, but as they reconstitute exactly there must be steric specificity. SVEDBERG and BROHULT<sup>12, 21</sup> found that the molecule is split in half (see Figure 4) when its solutions were irradiated with  $\alpha$ -particles and although the fragments have the same physical properties as those produced by dissociation with salts they are chemically modified

## EFFECT OF RADIATION ON MACROMOLECULES

since they cannot be reconstituted. They are of course formed under condition when the unirradiated molecule is in its associated state. This irreversible breakdown is referred to as 'splitting' in distinction to the reversible dissociation process. The splitting process was shown to be the result of the 'direct' action of the  $\alpha$ -particles since it occurred to exactly the same extent if the solution was irradiated at 20° C or frozen at -180° C. Quantitatively a molecule is split by the passage of a single  $\alpha$ -particle through it and this reaction is probably one of the earliest examples of a single-hit target process where the target corresponds to the physical dimensions of the molecule. As the probability that an ionization occurs in a given part of the molecule is extremely small, one must conclude that the highly specific splitting reaction can be brought about by energy absorbed anywhere within the molecule, *i.e.* that energy transfer occurs within the molecule.

The splitting of the haemocyanin molecule is the clearest example of the breaking-up of a relatively large structure by ionizing radiation and it would seem highly desirable to extend the investigations, for example, by determining the influence of the ion density of radiation\*, so as to establish whether this reaction is similar to the breaking of chromosomes. SVEDBERG and BROHULT<sup>12, 21</sup> did not find any changes in the sedimentation behaviour of serum albumin or haemoglobin when these were irradiated in solution at -180° C, though at 0° C extensive changes were produced (*cf.* next section). These proteins are therefore resistant to direct action although with very high doses—greatly in excess of those necessary for splitting haemocyanin—serum albumin<sup>20</sup> and fibrinogen<sup>23</sup> are chemically altered when irradiated dry.

*Indirect action*—The ultracentrifuge pattern of serum albumin and haemoglobin when irradiated with  $\alpha$ -rays at room temperature becomes polydisperse with components both heavier and lighter than the original material<sup>21</sup>. No such changes are brought about by dilute solutions of hydrogen peroxide and indicate that OH radicals are involved. BARRON and FINKELSTEIN<sup>24</sup> claim to have

\* PICKELS and ANDERSON<sup>22</sup> using a different haemocyanin (*Limulus polyphemus*) found a similar splitting into halves by x-rays. However, their results are contradictory; the observation that the actual amount of protein affected increased with concentration up to 15 per cent (*i.e.* the fraction of the protein split is nearly independent of the concentration) indicates that the free radicals produced in the water are not able to produce a split. On the other hand the addition of extraneous proteins greatly decreases the amount of splitting; this protective action suggests an indirect mechanism. However, the possibility that the added protein may influence the physicochemical processes involved in the dissociation of the haemocyanin and not the radiation response must not be overlooked. Unfortunately the decisive freezing experiment has not been carried out using x-rays.

#### CHANGES PRODUCED IN PROTEINS

found evidence for the formation of a homogeneous heavier component stated to be a dimer on irradiating serum albumin and serum globulin with x-rays. It would be interesting to know if the failure of BROHULT<sup>21</sup> to find this material is due to a difference between the reactions produced by x-rays and by  $\alpha$ -rays. When fibrinogen is irradiated with x-rays in solution<sup>25</sup> a polydisperse material having a higher sedimentation constant and increased viscosity is produced. The reaction was followed quantitatively by measuring the disappearance of the sedimentation peak due to the unchanged material. The kinetics of the process leave no doubt that the effect is produced by free radicals and this is confirmed by the fact that cysteine and thiourea act as protective agents.

Many other though less well-defined physical effects have been observed, such as changes in optical rotation, refractive index, surface tension and electrical conductivity<sup>26</sup>. Changes in viscosity are often observed though in some cases it is an increase and in others a decrease. For example, the viscosity of ovalbumen solution is increased if the irradiation is carried out at the isoelectric point or at lower pH values, but decreased at higher pH values<sup>27</sup>. Almost all proteins are denatured by ionizations, if this is defined as rendering the protein insoluble at the isoelectric point. This aspect has been very thoroughly studied and reviewed by FRICKE<sup>28</sup>. BARRON and FINKELSTEIN<sup>29</sup> observed that a solution of 0.7 per cent serum albumin is rendered insoluble when irradiated at 25° C, but not when irradiated at 3° C, but that this latter solution precipitates when raised subsequently to 25° C. Although an explanation in chemical terms has not been given this experiment may throw light on the observation that the results of a lethal dose of x-rays on frogs can be delayed indefinitely by freezing the animal (Chapter 6). Most of the data is consistent with the view that the free radicals produced in water bring about a change in shape of the protein molecule (*e.g.* unfolding) and thereby facilitate subsequent aggregation processes, giving rise to structures of increased molecular weight. In addition some main-chain degradation to give smaller products must also take place. The formation of definite dimers could possibly be brought about by the oxidation of SH groups to give an intermolecular disulphide bond, but there is no evidence that such a reaction does occur. No experiments have been carried out from which it can be decided which of the radicals are responsible for bringing about the different physical changes. The equally effective inactivation of non —SH enzymes under deaerated and aerated conditions suggests that OH radicals play a predominant part in all these reactions<sup>29, 31</sup>.

### EFFECT OF RADIATION ON MACROMOLECULES

The u.v. absorption spectrum of proteins is changed by irradiation although there is some disagreement in the experimental results<sup>27</sup>. BARRON *et al.*<sup>24</sup> found for serum albumin and globulin distinct increases at 2400 Å and 2800 Å (see Figure 5). Changes in other parts of the spectrum appeared less clear-cut. The chromophore giving rise to the absorption at 2400 Å in proteins has not been identified, but is believed to be the peptide bond. There is general agreement that the absorption maximum at 2790 Å is due to the aromatic amino-acid residues, tyrosine, phenylalanine and tryptophane, and the

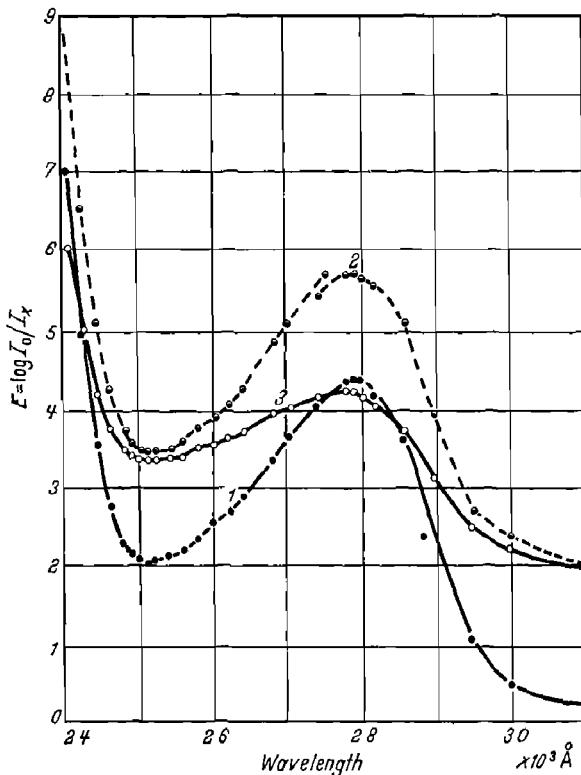


Figure 5. Effect of irradiation with X-rays on the absorption spectrum of bovine serum albumen, ( $10^{-5} M$ )<sup>24</sup>: (a) not irradiated; (b) irradiated with  $5 \times 10^4$  r; (c) irradiated with  $10^5$  r

increased intensity indicates a reaction with these groups. A complicating factor in all these experiments is that part of the protein is rendered insoluble and will scatter light and thereby give rise to an apparent increase in absorption. Since the amount of light scattered varies inversely as the fourth power of the wavelength this effect will be twice as great at 2400 Å as at 2800 Å. The change at 2400 Å may not represent a true increase in absorption and scattering is probably at least partly responsible; the finding that the absorption at this wavelength increased on standing could be inter-

## CHANGES PRODUCED IN PROTEINS

preted as a coagulation of insoluble protein to form large particles which scatter more.

In view of the importance to radiobiology of understanding the changes produced by irradiating proteins it is unfortunate that no definite chemical data is available. It would be most interesting to determine what changes are produced by irradiation in the amino-acid composition and in the titration curve of proteins. By analogy with the synthetic polymers (see p. 127) a large proportion of the energy would be expected to be concentrated by a transfer mechanism in the aromatic amino-acid residues (*i.e.* the proportion of these amino-acids changed by a given dose will be greater when they are incorporated in proteins than when they are irradiated as pure compounds).

## INACTIVATION OF ENZYMES

Much of the earlier work on the inactivation of enzymes in solution is of doubtful value, as the preparations used contained impurities which may have exercised a protective effect. The discovery by DALE<sup>34</sup> that added substances reduced the inactivation by x-rays of the enzymes carboxypeptidase and D-amino-acid-oxidase in dilute solution focussed attention on the role of indirect action in biological systems (see p. 48). The efficiency of inactivation of enzymes by indirect action is very much less than that by direct (see Chapter 2). *Table IV* shows that for many enzymes between

*Table IV. Yields of Enzymes inactivated by x-irradiation<sup>33</sup>*

<i>Enzyme</i>	<i>Yield 'G' (100 eV)</i>
<i>Yeast alcohol dehydrogenase</i>	3.4
<i>Phosphoglyceraldehyde dehydrogenase</i>	2.9
<i>Carboxypeptidase</i>	0.55
<i>D-Amino-acid-oxidase</i>	0.31
<i>Ribonuclease</i>	0.09
<i>Trypsin</i>	0.077
<i>Lysozyme</i>	0.03
<i>Catalase</i>	0.009

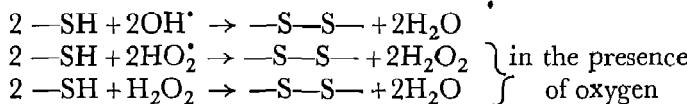
ten to one hundred ionizations are necessary to inactivate one enzyme molecule in dilute aqueous solution while by direct action one ionization per molecule is sufficient. The low yield for indirect action can either be the result of an extremely low affinity of the protein for the radicals so that radical recombination occurs preferentially, or due to the fact that there are many parts of the protein

### EFFECT OF RADIATION ON MACROMOLECULES

molecule which can react without however producing a change in biological activity (compare p. 62). The former possibility is ruled out since the ionic yield is independent of concentration above a minimum value<sup>31</sup>. Ribonuclease and carboxypeptidase solutions are inactivated to the same extent in the presence as in the absence of dissolved oxygen and this indicates that OH radicals are responsible. In support of this view it was found that chemically produced OH radicals also inactivate these enzymes<sup>31</sup>.

According to FORSSBERG<sup>32</sup> catalase is inactivated in solution by H radicals since irradiation is more effective in the absence of dissolved oxygen; however, chemically produced hydrogen atoms<sup>32a</sup> failed to bring about inactivation. Unlike all the other enzymes studied, the inactivation of catalase appears to depend on dose rate. The position concerning this enzyme is unsatisfactory and its behaviour to radiation should be investigated more fully.

The inactivation of the two dehydrogenases, SH enzyme (see *Table IV*), has a high ionic yield. Barron found that all SH enzymes were readily inactivated by x-rays and that dissolved oxygen powerfully enhances the action: BARRON<sup>33</sup> formulated inactivation as being due to the oxidation by the following reactions of the SH groups:



*Table V. Inhibition of Phosphoglyceraldehyde dehydrogenase and Adenosinetriphosphatase by x-rays; reactivation with Glutathione (added after x-irradiation<sup>33</sup>)*

Enzyme	x-ray dose (r)	Inhibition (%)	Reactivation (%)
<i>Phosphoglyceraldehyde dehydrogenase</i>	100	21	Complete
	200	50	62
	300	80	—
	500	94	10
	1000	73	22
<i>Adenosinetriphosphatase</i>	100	27	97
	500	41	56
	1000	73	—

In support of this series of reactions it is found that many of the enzymes can be reactivated, in part at least (see *Table V*) by the addition of glutathione which reduces the disulphide bonds back to SH groups. The experiments of Dale and others (see p. 114)

## INACTIVATION OF ENZYMES

indicate that this formulation of the oxidation of SH groups is a great over-simplification and that many other reactions occur simultaneously. Moreover the ionic yield for SH enzymes is close to one whereas if the above set of reactions applied it should be four. There can however be little doubt that oxidation of the —SH groups plays an important part in the inactivation of sulphhydryl enzymes and that the relatively high yield is due to the greater affinity of free radicals for SH groups than for other groups in the proteins. This means that these groups which are vital for activity react preferentially while in the other enzymes the essential groups compete less effectively for the free radicals. Oxidation of vital —SH groups to disulphide bonds is however unlikely to be of biological importance as in general the reducing capacity of the body is sufficient to reverse this reaction immediately (see Chapter 10).

Many enzymes carry a non-protein prosthetic group which though essential for activity can be reversibly detached from its specific protein. DALE<sup>34</sup> found that for D-amino-acid-oxidase the specific protein and the alloxazine adenine dinucleotide were approximately equally radiosensitive and the total amount of inactivation was additive when each component was irradiated individually and afterwards mixed. When solutions of the whole enzyme are irradiated the protein moiety and not the prosthetic group are destroyed; possibly the latter, being held within the protein, is sterically inaccessible to the free radicals. Biologically active materials chemically related to these prosthetic groups (*e.g.* diphosphopyridine nucleotide and adenosine triphosphate) are destroyed by x-rays in aqueous solutions though with low yields<sup>33</sup>. An important non-peptide structure found in many biologically active proteins is the porphyrin ring present for example in catalase, peroxidases, haemoglobin and cytochrome-c. A characteristic property of this substance is a strong absorption band in the visible region at 4100 Å (the Soret band) which is responsible for its red colour. FRICKE<sup>35</sup> showed that this absorption was reduced when haemoglobin was irradiated in aqueous solution\*; BARRON and FLOOD<sup>36</sup> have recently carried out a more detailed investigation and state that the decrease in absorption is linear with dose and compute an ionic yield of 0.05, although it is doubtful what meaning can be attached to this value.

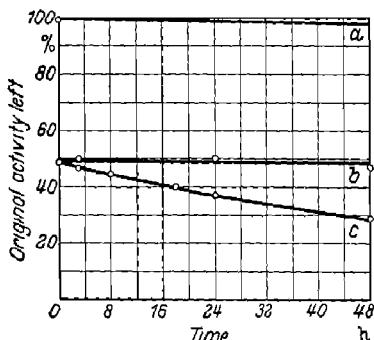
The recent observation, that the inactivation of trypsin<sup>38</sup> and pepsin<sup>39</sup> continued after irradiation has been concluded (see

---

\* Direct action is unable to affect the chromophoric groups of porphyrin and the absorption maxima is unaffected even when there have been an average of four ionizations per molecule<sup>37</sup>.

## EFFECT OF RADIATION ON MACROMOLECULES

*Figure 6)* may be of biological significance. The after-effect is temperature-dependent and differs in this respect from the degradation of nucleic acid which also continues after irradiation (see p. 148). The small quantity of hydrogen peroxide which is formed during irradiation cannot be responsible and it seems likely that the decom-



*Figure 6. Continual slow inactivation of trypsin after irradiation (2,500 r of x-rays,  $4 \times 10^{-3}$  per cent trypsin, in  $5 \times 10^{-3}$  N  $\text{H}_2\text{SO}_4$ ) if soln is stored at 25° C. At 2° C no post-effect can be detected: (a) Trypsin unirradiated and kept at 2° and 25° C; (b) Trypsin irradiated and kept at 2° C; (c) Trypsin irradiated and kept at 25° C*

position of an unstable intermediary is involved. It would be interesting to determine if this after-effect were related to the delayed precipitation phenomena of proteins irradiated at low temperatures (see p. 137).

## REACTIONS OF NUCLEIC ACIDS\*

*Structure of nucleic acids*—There are a number of excellent reviews on this subject<sup>40</sup> and only a brief summary will be given. Nucleic acids are macromolecules of very high molecular weight carrying recurrent negative charges and consequently fall into the class of polyelectrolytes<sup>16</sup>, the general properties of which, particularly in aqueous solution, are now being intensively investigated. RNA and to a lesser extent DNA, are very readily broken up and even now one cannot be certain that they have been extracted without degradation. The modern methods used for obtaining DNA seem to be satisfactory since they yield a biologically active preparation—the transforming principles—when applied to bacteria. Although few workers believe that the DNA isolated from whole cells is a homogeneous product, techniques for separating the mixture into different components have only recently been discovered<sup>44</sup>.

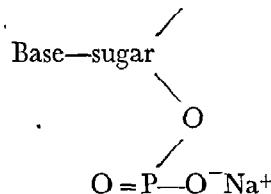
The chemical constitution of the nucleic acids has been well

---

\* The sodium salts of deoxyribosenucleic acids will be abbreviated to DNA and those of ribosenucleic acid to RNA.

## REACTIONS OF NUCLEIC ACIDS

established and it is known that they are built up on the general pattern



although there are conflicting views concerning the existence of branched chains. The two classes RNA and DNA differ in the sugars they contain and the attachment of the various groups to the ribose and deoxyribose is now known with some certainty<sup>41</sup>. The other constituents are purine and pyrimidine bases; and most nucleic acids contain four different kinds together with one or possibly two others in low concentration. The order in which these bases are arranged is not known, but a start has been made into investigating this extremely difficult problem by SMITH and MARKHAM<sup>42</sup> who have isolated some di- and tri-nucleotides. There can be no doubt that the different bases are not present in a regular sequence<sup>43</sup> and there is some evidence that there are short lengths along the chain of some DNAs which are made up entirely of purines.

Much less is known about the size and shape of these molecules. DNA can be extruded as fibres when it gives an excellent x-ray diffraction pattern which together with the exceptionally high density of the dry material led ASTBURY<sup>45</sup> to propose a close packed structure in which the sugars and bases were stacked in layers on top of one another with the phosphate groups in between. In the light of more recent data this model has had to be modified though many features have been retained. The twin spiral proposed by WATSON and CRICK<sup>46</sup> shown in *Figure 7* is consistent with the available x-ray data and has considerable biological interest since it appears to provide a model for the synthesis of genetic material based on a template principle. It must be stressed that this double molecule structure is not proved and it is possible that other stereochemical arrangements will be found which also fit the known facts.

DNA solutions show many anomalous properties, such as non-Newtonian viscosity (see p. 132) which are due to molecular interaction and make the determination of the molecular dimensions extremely difficult. Most of the conventional methods such as sedimentation, diffusion and viscosity cannot be used and one of the few methods available is that of light scattering<sup>47, 48</sup>. This shows that the molecular weight of undegraded DNA particles in solution

## EFFECT OF RADIATION ON MACROMOLECULES

varies between  $6-10 \times 10^6$ . These molecular weight determinations agree with the value of  $6 \times 10^6$  found for the size of the *pneumococcus* transforming principle<sup>49</sup> from target determination (see p. 60). According to light-scattering data the molecule exists in dilute salt solutions as an asymmetric coil of about 6000 Å. The dimensions of this coil are determined by the electrical repulsion of the charged groups and will therefore vary if the pH and salt concentration of the solution is changed (compare behaviour of polymethacrylic acid<sup>16</sup> p. 132). Other measurements, notably flow birefringence<sup>50</sup>, lead to the opposite conclusion that the DNA molecule exists in solution as a rigid rod. At present it is impossible to reach any definite conclusions regarding this point.

On adding urea to solutions of DNA the molecular weight, as determined by light-scattering, is reduced almost exactly to half<sup>51</sup>.

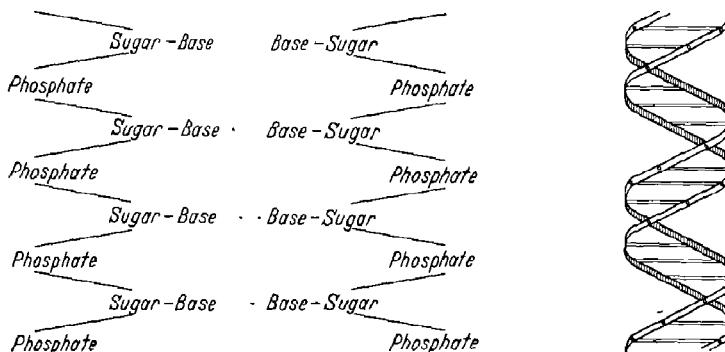


Figure 7. The twin-spiral structure of two DNA chains proposed by WATSON and CRICK, on x-ray evidence

As urea has been shown to break-up molecular aggregates of dyes<sup>52</sup> and of proteins<sup>53</sup> which are held together by hydrogen bonds, it would appear as if DNA exists in solution as a double molecule held together by these bonds. We do not know if there is any correlation between this observation and the postulated twin-spiral structure.

The data for RNA is very scarce. Its molecular weight was believed to be of the order of  $5 \times 10^4$  but recent investigations<sup>71</sup> indicate that the value is of the order of  $5 \times 10^5$  for undegraded samples.

*The structure of nucleoproteins*—There are at least two distinct types of nucleoproteins; those in which DNA is combined with basic proteins by salt links—these may be called *protein nucleates*—and the much larger and less well-defined class of viruses and liver nucleoproteins<sup>40</sup>. The viruses range from the relatively simple, crystalline, plant viruses to complex structures which can more properly

## REACTIONS OF NUCLEIC ACIDS

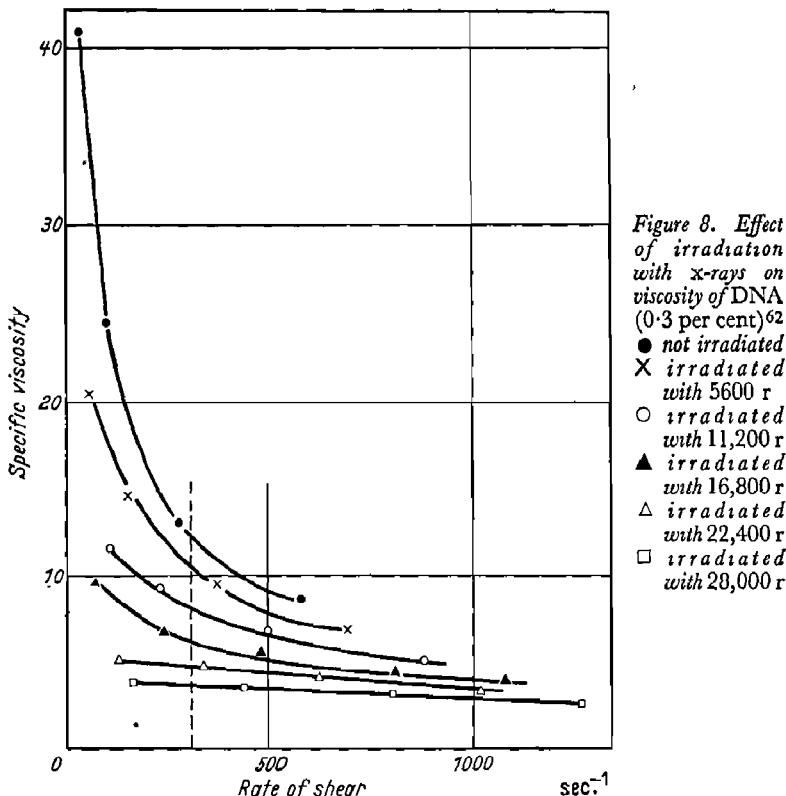
be considered as organisms since they have a morphological structure. The viruses contain from 5 to 50 per cent nucleic acid which may be of either type. The proteins are not joined to the nucleic acid by a large number of salt links as in the nucleates and the type and the firmness of the attachment varies from one virus to another. Complete separation is rarely possible without degradation.

The nucleates are of particular interest in radiobiology since they are believed to make up at least a part of the chromosomes. Nucleates can be obtained from the nuclei of cells in high yield and a very pure source is the spermheads of fish<sup>54</sup> which are entirely composed of the complex between DNA and the very low molecular weight proteins known as protamines. In the nuclei of mammalian cells DNA is associated with histones, which are more complex than the protamines but still relatively simple proteins. Both histones and protamines contain a preponderance of the basic amino-acids lysine and arginine. These two amino-acids make up 80 per cent of the protamines which can be considered as mixed polypeptides of the basic amino-acids. There exists considerable confusion concerning the properties of the nucleates since different products are obtained by different methods of extraction. When cell nuclei are extracted with water a solution of nucleate is obtained which has a low viscosity<sup>55</sup>. On the addition of salt this is precipitated and the complex is completely insoluble in 0.1M salt<sup>56</sup>. In concentrated salt solutions the nucleate dissolves again to give a highly viscous solution, from which by the addition of water, a white fibrous precipitate is obtained which has exactly the same composition as the original nucleate from which it differs, however, in being quite insoluble in water<sup>57</sup>. From reconstitution experiments with DNA and protamine ALEXANDER<sup>58</sup> concluded that in the original water soluble material the protamine molecules are lined up with one DNA molecule by salt links. The polyelectrolyte character of the DNA is suppressed and the complex can assume a compact configuration giving solutions of low viscosity. In strong salt solutions the salt bridges between the basic side chains of the protamine and the ionized phosphate groups of the DNA are broken and the two molecules have an independent existence. When the salt concentration is lowered the two interact in a non-regular manner to give a disordered precipitate which in its arrangement is quite different from the water soluble starting material.

Although it has been known<sup>55</sup> since 1899 that protein nucleates are water-soluble many investigators have isolated an insoluble precipitate by the MIRSKY<sup>56</sup> procedure which consists of extracting the tissue with 0.1M salt to remove extraneous protein, dissolving in

### EFFECT OF RADIATION ON MACROMOLECULES

2M salt and collecting the precipitate formed on dilution. This simple method is very effective in providing uncontaminated DNA and protamine (or histone) but does not give the conjugate present in the cell. This must be isolated by extraction with water when difficulties may be encountered in removing cytoplasmic material. In all the DNA-protamine complexes isolated, the ratio of basic amino-acids to anionic phosphate groups has been close to, but slightly less than one (*i.e.* the complex is made up approximately of



65 per cent DNA and 35 per cent protamine). The protein nucleate consists therefore of a macromolecule carrying a small negative charge which is responsible for its solubility in water<sup>58</sup>. The nucleo-histones are much more complicated and no clear pattern of the amount of DNA to histone has yet become apparent.

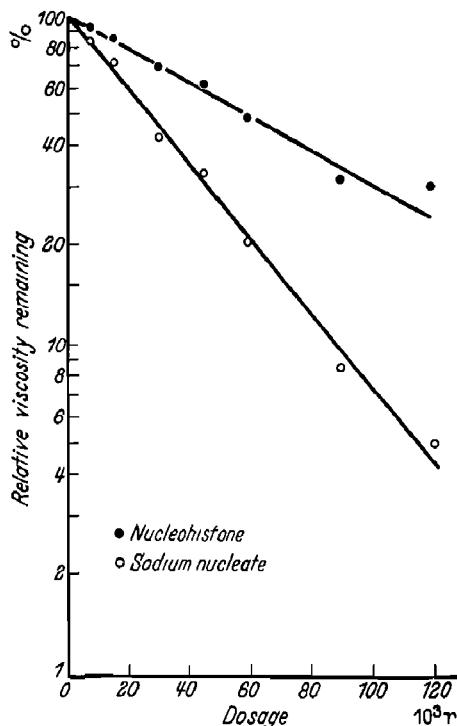
The problem of the constitution of the chromosomes has not been solved; as all cell nuclei contain large quantities of protein nucleate it is tempting to identify these as chromosomal materials. On the other hand most cell nuclei also contain other proteins and it has been suggested that the chromosomes are protein fibres with adher-

## REACTIONS OF NUCLEIC ACIDS

ing nucleic acids. For fish (the spermheads of which are made up entirely of nucleate) the latter suggestion can be dismissed but it is clearly not possible to generalize from this to mammalian chromosomes. The absence of all analytical data concerning the chromosomes is one of the most remarkable omissions of contemporary biochemistry and bears testimony to the difficulty of dealing with these structures.

*Depolymerization by x-rays*—One of the most characteristic and most readily measured properties of solutions containing DNA is their high viscosity; this decreases with increasing rate of shear (see p. 132).

*Figure 9. Logarithmic relationship between dose and decrease in viscosity for 0.1 per cent solns of DNA irradiated with x-rays. The upper curve is for the degradation of thymus nucleoprotein indicating the protective effect of the protein<sup>60</sup>*



On irradiation with x-rays, either in solution or dry, this viscosity is greatly decreased (see *Figure 8*). A comparison by KOENIG and PERRINGS<sup>59</sup> shows that the indirect effect is less efficient than the direct effect (*i.e.* the energy per unit weight of DNA to produce a given change in viscosity or sedimentation is less for the dry material than for the material in solution). SPARROW and ROSENFIELD<sup>60</sup> were the first observers to record the decrease in viscosity on irradiating DNA solution with x-rays and found, as did G. C. BUTLER<sup>61</sup>, a logarithmic relationship between the viscosity at high shear rates and the radiation dose (see *Figure 9*). A nucleohistone in 2M salt

#### EFFECT OF RADIATION ON MACROMOLECULES

showed a much smaller loss in viscosity<sup>60</sup> than did DNA; as in the salt solution used the nucleohistone was fully dissociated the protective action of the histone was almost certainly due to competition for the degrading radicals produced in water. Similar protection effects have been observed with serum albumin<sup>62</sup>, glucose<sup>61</sup>, methanol<sup>61</sup>, thiourea<sup>63</sup> and cysteamine<sup>64</sup>.

TAYLOR, GREENSTEIN and HOLLOWENDER<sup>62</sup> made the significant observation that the viscosity continued to fall for many hours after the irradiation was stopped and that the magnitude of this effect was almost independent of temperature (see *Figure 10*). It is difficult to decide how much of the observed decrease in viscosity occurs immediately on irradiation since the after-effect will start while the irradiation is still in progress. J. A. V. BUTLER<sup>65</sup> claims that there are two distinct effects, an initial reduction followed by the much larger after-effect. TAYLOR *et al.*<sup>62</sup> on the other hand found no evidence for an immediate effect, *i.e.* they extrapolated the rate curves shown in *Figure 10* to zero degradation at zero time. J. A. V. Butler used much lower dose rates and the so-called initial effect may therefore have been an 'after-effect' which took place during the irradiation. The observation by G. C. BUTLER<sup>61</sup> that there is a very rapid fall in viscosity immediately after irradiation supports the view that there may be no distinct initial effect.

CONWAY and BUTLER<sup>64, 65</sup> claim that added chemicals can only reduce the degradation of DNA if added before the irradiation and that the after-effect is not influenced if the protective agents are added after the irradiation. ERRERA<sup>79</sup> on the other hand finds (see *Figure 11*) that glutathione can almost completely prevent the slow decrease in viscosity even when added *after* the irradiation with x-rays. This finding is of great significance and must be considered in any theory advanced for the mechanism of the after-effect.

The evidence concerning the role of oxygen in the viscosity reduction is also contradictory. The initial experiments<sup>60, 62</sup> were carried out in aerated solution. In a subsequent detailed and quantitative study G. C. BUTLER<sup>61</sup> claimed that the decrease in viscosity was the same in aerated as in carefully deoxygenated solutions. A year later, J. A. V. BUTLER<sup>65</sup> found the exact reverse; in the absence of oxygen there was no after-effect although the ill-defined initial effect was claimed to be the same in solutions saturated with nitrogen as with air. In contradiction to all the previous workers DANIELS, SCHOLES and WEISS<sup>66</sup> find that the initial effect on the viscosity is larger in the absence of oxygen but the after-effect smaller. Their definition of the initial effect, however, is particularly unsatisfactory

## REACTIONS OF NUCLEIC ACIDS

as their first measurement was made two hours after irradiation, a time interval in which G. C. Butler finds that most of the after-effect has already occurred.

In the present confused state the outstanding experimental work of Miss T. ALPER<sup>67</sup> on the inactivation of bacteriophages whose active principle is DNA may throw some light on the nature of the

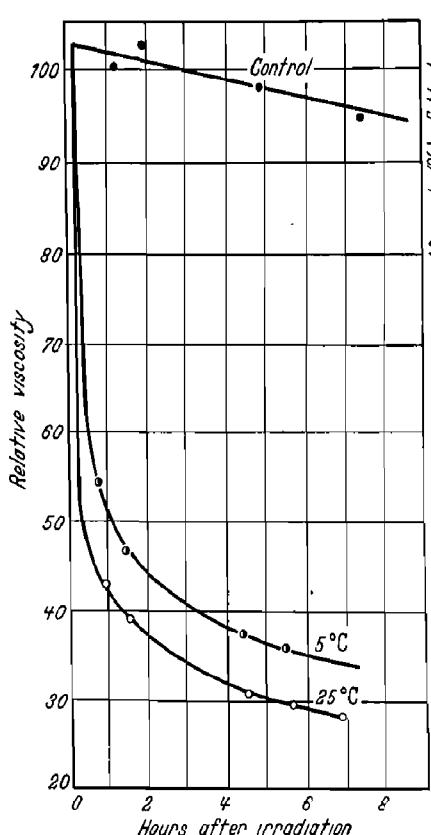


Figure 10. Decrease in viscosity of DNA after irradiation with x-rays (56,000 r) has been finished. Effect of temperature on the after-effect is shown<sup>62</sup>

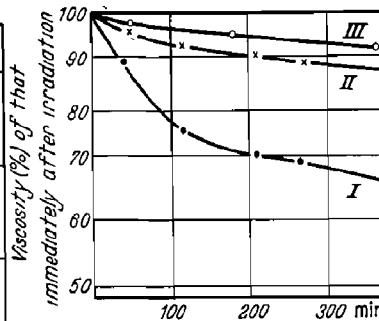


Figure 11. Action of glutathione in preventing the slow decrease in viscosity of DNA which continues after irradiation with x-rays. I No glutathione added after the irradiation; II 0.05M glutathione added after the irradiation; III 0.05M glutathione added to non-irradiated DNA soln<sup>79</sup>

after-effect. Phages are inactivated to a greater extent in oxygen-free than in oxygenated dilute solutions, where the indirect effect predominates (*cf.* Chapter 8). Inactivation continues in aerated solutions after irradiation and this was shown to be due to hydrogen peroxide formed in the solution. The susceptibility of phages to hydrogen peroxide was shown to be greatly increased by prior irradiation. Although the viscosity of pure DNA was not changed by low concentration of peroxide after irradiation, DNA-like phages become

#### EFFECT OF RADIATION ON MACROMOLECULES

more sensitive to this reagent<sup>68</sup>. If hydrogen peroxide plays a significant part in the reaction leading to a loss in viscosity the contradictory results may be explained. As was shown in Chapter 3 the amount of hydrogen peroxide formed by irradiation is influenced by the solutes present. With a reactive solute which combines readily with free radicals, hydrogen peroxide may be produced even in the absence of oxygen<sup>69</sup> because the 'back reaction' is eliminated. In the presence of oxygen the quantity of hydrogen peroxide formed can be enhanced or reduced by the presence of impurities (see p. 109). Small differences in technique and in the purity of the DNA preparations used can therefore bring about large changes in the amount of hydrogen peroxide formed.

The present confusion is unfortunate since it would be interesting to know the correlation—if any—between the effect of x-rays on DNA *in vitro* and chromosome breakage. The first essential in this connection is to clarify the nature of the oxygen effect *in vitro*.

Equally difficult is the interpretation of the changes in viscosity in molecular terms. In simple systems the viscosity of a high polymer is directly related to its molecular weight. With polyelectrolytes, however, the viscosity is both a function of the shape and size of the molecule and since DNA solutions are non-Newtonian (see p. 132) interaction between molecules is a further contributing factor<sup>16</sup>. In such a system ALEXANDER and Fox<sup>14</sup> demonstrated that identical changes in viscosity could be produced by internal crosslinking to give a less asymmetric structure as by main-chain degradation.

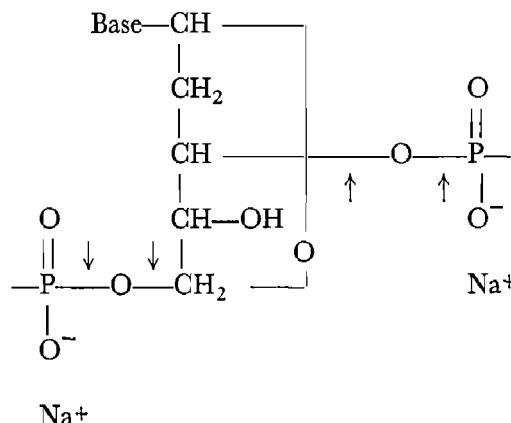
The sedimentation studies of TAYLOR *et al.*<sup>62</sup>, subsequently confirmed in several other laboratories, leave little doubt that x-rays depolymerize DNA by breaking main-chain bonds. No reliable quantitative values have been obtained for the molecular weight changes produced since molecular inter-action makes a quantitative evaluation of the sedimentation results impossible. G. C. BUTLER<sup>61</sup> estimates that the ionic yield M/N for the main-chain breakdown of DNA is extremely low and quotes an upper value of 0·02. As in the case of enzyme inactivation this does not mean that DNA does not react with free radicals—indeed the experiments of DALE<sup>34</sup> show that it competes very effectively for these—but that only few reactions lead to main-chain breakdown.

The effect of x-rays on RNA has not been studied nearly so extensively. GRINNAN and MOSHER<sup>71</sup> report that a sample isolated from liver nucleoprotein behaves similarly to DNA in that the viscosity continues to decrease after the irradiation has been stopped.

*Chemical changes produced by x-rays*—No data is available of the chemical effect of ionizing radiations on dry DNA. As, however,

### REACTIONS OF NUCLEIC ACIDS

one ionization per phage is sufficient for inactivation (see p. 58) it must be concluded that direct action is extremely efficient in disrupting DNA molecules. The changes brought about by the free radicals formed in aqueous solution have been studied by a number of workers. If there is an immediate effect of x-rays on the viscosity depolymerization can only occur if one of the bonds



indicated by an arrow is broken. Reaction with the purine or pyrimidine basis or with the sugar ring would not decrease the molecular size directly. From experiments with a synthetic polyphosphate (see p. 134) we know that the  $-\text{P}-\text{O}-$  bond is extremely radiation resistant, while reaction at a carbon bond in sugars occurs readily<sup>72</sup>. Of particular interest in this connection is the observation of SCHNEIDERMAN<sup>73</sup> and his colleagues that the high molecular weight polysaccharide, hyaluronic acid is depolymerized in solution by irradiation with relatively low doses of x-rays and the process continues after the radiation is complete (see *Figure 12*). Molecular weight determinations by light-scattering proved unambiguously that during the after-effect depolymerization continues. These experiments indicate by analogy that the  $-\text{O}-\text{C}-$  main-chain bond is the one attacked in DNA. SCHOLES and WEISS<sup>74</sup> have obtained some evidence that the sugar ring is oxidized and that this renders the adjacent ester-main-chain bond more susceptible to hydrolysis. Such a reaction would provide a satisfactory mechanism for the after-effect in both DNA and hyaluronic acid.

TAYLOR *et al.*<sup>62</sup> failed to notice any chemical changes after irradiations sufficient to reduce the viscosity of DNA to a small fraction of its original value. No inorganic phosphate or dialysable products were formed and the u.v. absorption spectrum was unchanged. After exposures to high doses the u.v. spectrum undergoes

### EFFECT OF RADIATION ON MACROMOLECULES

minor changes<sup>73</sup> and there is evidence that every part of the DNA and RNA molecule is attacked<sup>74</sup>. Deamination with evolution of ammonia, ring opening of the heterocyclic bases, fission of the glycoside linkages, breaking of the ester link with the formation of inorganic phosphate and an increase in titrable groups have all been observed. SCHOLES and WEISS<sup>74</sup> believe that the presence of oxygen favours deamination and retards the formation of inorganic phosphate. There is no evidence for the marked predominance of any one reaction and this may explain the low ionic yield for main-chain breakdown.

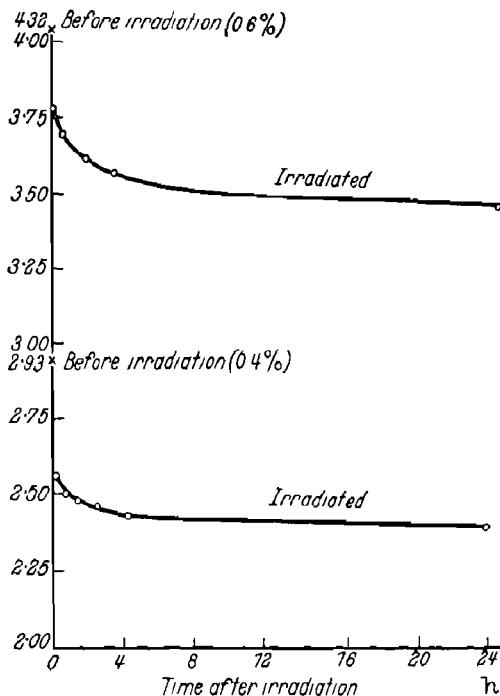


Figure 12. Effect of irradiation with 9000 r x-rays on the viscosity of hyaluronic acid solns. There is both an initial and a very much smaller slow after-effect<sup>73</sup>

*Effect of x-rays on nucleoproteins*—Although the inactivation of viruses by ionizing radiations has been extensively investigated (see Chapter 2) relatively little attention has been paid to the chemical and physical changes produced. The rigidity of a nucleoprotein gel extracted from chicken erythrocytes is significantly reduced (see Figure 13) by relatively small doses of x-rays<sup>75</sup> which would only slightly reduce the viscosity of DNA solutions of comparable concentration. ERRERA<sup>75</sup> found a greater decrease in rigidity if the cell was irradiated and the nucleoprotein gel extracted afterwards. ROLLENAAL *et al.*<sup>76</sup> obtained a partial dissociation of a nucleohistone

## REACTIONS OF NUCLEIC ACIDS

on irradiating this in aqueous suspension and detected histone in solution after the irradiation. A possible interpretation is that the affinity of DNA for histone is reduced. If this is the case there is an unexpected parallelism between the action on DNA of x-rays and of the radiomimetic alkylating agents (see Chapter 7) which reduce the protamine combining capacity of DNA<sup>77</sup>.

*Effect on DNA in vivo*—The depolymerization of nucleic acids by x-rays has not been unequivocally proved to take place *in vivo*, though several investigations suggest that deoxyribonucleic acid may be more radiosensitive *in vivo* than *in vitro*. FEINSTEIN and

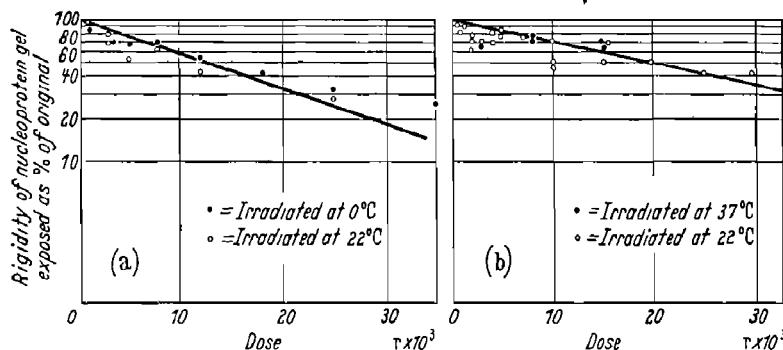


Figure 13. Effect of irradiation with x-rays on the rigidity of a nucleoprotein gel extracted from the erythrocytes of chickens: (a) erythrocytes irradiated and nucleoprotein gel extracted after irradiation; (b) irradiation of nucleoprotein gel *in vitro*<sup>73</sup>

C. L. BUTLER<sup>78</sup> have isolated from the rat's intestine a fraction rich in nucleoproteins, in which the phosphorus content is considerably diminished after irradiation of the whole animal with 800 r. LIMPEROS and MOSHER<sup>63</sup> observed two effects of irradiation on the DNA of the rat's thymus: (i) if the animal is killed immediately after irradiation, the nitrogen content of the DNA is low as a result of a loss in purine bases; (ii) the DNA extracted from the thymus 24 hours after irradiation is almost completely depolymerized. Thiourea, a protector against the lethal effects of ionizing radiations (see Chapter 14), does not prevent the first effect, but partly inhibits the second.

## REFERENCES

- <sup>1</sup> ALEXANDER, P., CHARLESBY, A. and ROSS, J., *Proc. roy. Soc.*, 1954, **223A**, 392
- <sup>2</sup> CHARLESBY, A., *ibid.*, 1952, **215A**, 187
- <sup>3</sup> CHARLESBY, A., *Atoms*, 1954, **5**, 12
- <sup>4</sup> CHARLESBY, A., *J. Polym. Sci.*, 1953, **6**, 521
- <sup>5</sup> ALEXANDER, P. and CHARLESBY, A., Unpublished

EFFECT OF RADIATION ON MACROMOLECULES

- 6 ALEXANDER, P. and CHARLESBY, A., see *Brit. Emp. Cancer Camp. Annu. Rep.*, 1954, **31**, 17
- 7 CHARLESBY, A., *Atomic Energy Res. Est. Rep. No. M/R 978*, 1952
- 8 LAWTON, E. J., BUECHE, A. M. and BALWIT, J. S., *Nature, Lond.*, 1953, **172**, 76
- 9 CHARLESBY, A., *Proc. roy. Soc.*, 1954, **222A**, 60
- 10 MANION, J. P. and BURTON, M., *J. phys. Chem.*, 1952, **56**, 560
- 11 ALEXANDER, P. and CHARLESBY, A., *Nature, Lond.*, 1954, **173**, 578
- 12 SVEDBERG, T. and BROHULT, S., *ibid.*, 1939, **143**, 938
- 13 LEA, D. E., *Actions of Radiations on Living Cells*, p. 100, Cambridge, 1947
- 14 ALEXANDER, P. and FOX, M., *Nature, Lond.*, 1952, **169**, 572
- 15 ALEXANDER, P. and FOX, M., *J. Chim. phys.*, 1953, **50**, 415
- 16 ALEXANDER, P. and HITCH, S. F., *Biochim. biophys. Acta*, 1952, **9**, 218
- 17 ALEXANDER, P. and FOX, M., *Trans. Faraday Soc.*, 1954, **50**, 605
- 18 ASTBURY, W. T. and WOODS, H. J., *Phil. Trans.*, 1933, **232A**, 333
- 19 ALEXANDER, P. and HUDSON, R. F., *Wool, its Chemistry and Physics*, Chapman and Hall, London, 1954, p. 68
- 20 ALEXANDER, P. and CHARLESBY, A., unpublished
- 21 BROHULT, G., *Nova Acta Soc. Sci. upsal.*, 1940, **12**, No. 4
- 22 PICKELS, E. G. and ANDERSON, R. S., *J. gen. Physiol.*, 1946, **30**, 83
- 23 KOENIG, V. L. and PERRIN, J. D., *Arch. Biochem. Biophys.*, 1952, **38**, 105
- 24 BARRON, E. S. G. and FINKELSTEIN, *ibid.*, 1952, **41**, 212
- 25 SHERAGA, H. A. and NIMS, L. F., *ibid.*, 1952, **36**, 336
- 26 ARNOW, L. E., *Physiol. Rev.*, 1936, **16**, 671
- 27 ARNOW, L. E., *J. biol. Chem.*, 1935, **110**, 43
- 28 FRICKE, M., *Cold Spr. Harb. Sym. quant. Biol.*, 1938, **6**, 164
- 29 HOLMES, B., *Nature, Lond.*, 1950, **165**, 266
- 30 DALE, W. M., GRAY, L. H. and MEREDITH, *Phil. Trans.*, 1949, **242A**, 33
- 31 COLLINSON, E., DAITON, F. S. and HOLMES, B., *Nature, Lond.*, 1950, **165**, 267
- 32 FORSSBERG, A., *ibid.*, 1947, **159**, 308
- 32a SUTTON, H. C., *Disc. Faraday Soc.*, 1952, **12**, 281
- 33 BARRON, E. S. G., *Symposium on Radiobiology*, Wiley, New York, 1952, p. 216; *Radiation Res.*, 1954, **1**, 18
- 34 DALE, W. M., *Biochem. J.*, 1942, **36**, 80
- 35 FRICKE, H., *Amer. J. Roentgenol.*, 1927, **17**, 611
- 36 BARRON, E. S. G. and FLOOD, V., *Arch. Biochem. Biophys.*, 1952, **41**, 203
- 37 APPLEYARD, R. K., *ibid.*, 1952, **40**, 611
- 38 MACDONALD, M. R., *Brit. J. Radiol.*, 1954, **27**, 63
- 39 ANDERSON, R. S., *ibid.*, 1954, **27**, 65
- 40 GREENSTEIN, J. P., *Advanc. Protein Res.*, 1944, **1**, 210  
BRACHET, J., *Actualités Biochimique*, No. 16, 1952, Liège
- DAVIDSON, J. N., *The Biochemistry of the Nucleic Acids*, Methuen, London, 1954
- DAVISON, P. F., CONWAY, B. E. and BUTLER, J. A. V., *Progr. Biophys.*, 1954, **4**, 148
- 41 BROWN, D. M., MICHELSON, A. M. and TODD, A. R., *Trans. Faraday Soc.*, 1954, **50**, 291

REFERENCES

- <sup>42</sup> SMITH, J. D. and MARKHAM, R., *Biochim. biophys. Acta*, 1952, **8**, 350
- <sup>43</sup> ZAMENHOF, S. and CHARGRAFF, E., *J. biol. Chem.*, 1950, **187**, 1
- <sup>44</sup> CHARGRAFF, E., CRAMPTON, C. F. and LIPSHITZ, R., *Nature, Lond.*, 1953, **172**, 289
- BROWN, G. L. and WATSON, M., *ibid.*, 1953, **172**, 339
- <sup>45</sup> ASTBURY, W. T., *Symp. Soc. exp. Biol.*, 1947, **1**, 66
- <sup>46</sup> WATSON, J. D. and CRICK, F. H. C., *Nature, Lond.*, 1953, **171**, 964
- <sup>47</sup> REICHMANN, M. E., BUNCE, B. H. and DOTY, P., *J. Polym. Sci.*, 1953, **10**, 109
- <sup>48</sup> POUYET, J. and SARDRON, C. H., *Trans. Faraday Soc.*, 1954, **50**, 303; *J. Polym. Sci.*, 1952, **9**, 531
- <sup>49</sup> FLUDE, D., DREW, R. and POLLARD, E. C., *Proc. nat. Acad. Sci., Wash.*, 1952, **38**, 180
- <sup>50</sup> SCHWANDER, H. and CERF, R., *Helv. chim. Acta*, 1951, **34**, 436
- <sup>51</sup> STACEY, K. A. and ALEXANDER, P., *Trans. Faraday Soc.*, 1954, **50**, 303
- <sup>52</sup> ALEXANDER, P. and STACEY, K. A., *Proc. Roy. Soc.*, 1952, **A212**, 274
- <sup>53</sup> STEINHARDT, J., *J. biol. Chem.*, 1948, **123**, 543
- <sup>54</sup> FELIX, K., FISCHER, H., KREKELS, A. and RAUEN, H. M., *Hoppe-Seyl. Z.*, 1950, **286**, 67
- <sup>55</sup> BANG, J., *ibid.*, 1899, **27**, 463
- <sup>56</sup> MIRSKY, A. E. and POLLISTER, A. W., *J. gen. Physiol.*, 1946, **30**, 117
- <sup>57</sup> STERN, K. G., *Exp. Cell Res. Suppl.*, 1949, **1**, 97
- <sup>58</sup> ALEXANDER, P., *Biochim. biophys. Acta*, 1953, **10**, 595
- <sup>59</sup> KOENIG, V. L. and PERRINGS, J. D., *U.S. Atomic Energy Rep. AECU 2319*
- <sup>60</sup> SPARROW, A. H. and ROSENFIELD, F. M., *Science*, 1946, **104**, 245
- <sup>61</sup> BUTLER, G. C., *Canad. J. Res.*, 1949, **B27**, 972
- <sup>62</sup> TAYLOR, B., GREENSTEIN, J. P. and HOLLOWENDER, A. E., *Arch. Biochem. Biophys.*, 1948, **16**, 19
- <sup>63</sup> LIMPEROS, G. and MOSHER, W. A., *Amer. J. Roentgenol.*, 1950, **63**, 691
- <sup>64</sup> CONWAY, B. E., *Brit. J. Radiol.*, 1954, **27**, 42
- <sup>65</sup> BUTLER, J. A. V. and CONWAY, B. E., *J. chem. Soc.*, 1950, 3418
- <sup>66</sup> DANIELS, M., SCHOLES, G. and WEISS, J., *Nature, Lond.*, 1953, **171**, 1153
- <sup>67</sup> ALPER, T., *Disc. Faraday Soc.*, 1952, **12**, 234
- <sup>68</sup> CONWAY, B. E. and BUTLER, J. A. V., *J. chem. Soc.*, 1952, 834
- <sup>69</sup> DAINTON, F. S. and SUTTON, H. C., *Trans. Faraday Soc.*, 1953, **49**, 1011
- <sup>70</sup> ALEXANDER, P. and FOX, M., *Nature, Lond.*, 1952, **169**, 572
- <sup>71</sup> GRINNAN, E. L. and MOSHER, W. A., *J. biol. Chem.*, 1951, **191**, 719
- <sup>72</sup> KENOKH, M. A., *J. gen. Chem., Moscow*, 1950, **20**, 1560
- <sup>73</sup> SCHOENBERD, M. D., BROOK, R. E., HALL, J. J. and SCHNEIDERMAN, H., *U.S. Atomic Energy Commission U.C.L.A.*, 83, 1950; *U.C.L.A.*, 11, 1949
- <sup>74</sup> SCHOLES, G. and WEISS, J., *Biochem. J.*, 1954, **56**, 65
- <sup>75</sup> ERRERA, M. C. R., *Cold Spr. Harb. Symp. quant. Biol.*, 1947, **12**, 60
- <sup>76</sup> ROLLENAAL, H. M., BILLAMY, W. D. and BALDWIN, T. N., *Nature, Lond.*, 1951, **169**, 694
- <sup>77</sup> ALEXANDER, P., *ibid.*, 1952, **169**, 226
- <sup>78</sup> FEINSTEIN, R. N. and BUTLER, C. L., *Proc. Soc. exp. Biol. Med.*, 1952, **79**, 181
- <sup>79</sup> ERRERA, M. C. R., *Bull. Soc. Chim. biol.*, 1951, **33**, 555

## CYTOTOLOGICAL AND GENETICAL PHENOMENA

### INTRODUCTION

BEFORE considering the chemical changes which are produced in the protoplasm and nuclei of irradiated cells and tissues it is necessary to describe the morphological effects produced by ionizing radiation. Physicians using x-rays and radium for clinical treatment first discovered the marked changes produced in tissues about fifty years ago and this attracted the attention of pathologists. This was the period in which biological lesions were described in great detail and the arrest of mitosis due to the action of x-rays clearly recognized. The study of these effects led BERGONIÉ and TRIBONDEAU<sup>1</sup> to formulate their famous law which forms the foundation of the radiotherapy of cancer\*. At this time, however, no one attempted to examine experimentally the two fundamental problems: (i) How is it that certain radiation effects produced in cells can be repaired while others persist and are transmitted to subsequent generations? (ii) What are the physicochemical phenomena within the cell which precede the onset of the visible lesions?

The answer to the first question had to await the outstanding researches of Muller who showed that ionizing radiations greatly increase the frequency of visible and heritable mutations, and who established them as mutagenic agents. Following on this work with *Drosophila*, induced mutations were observed in all living matter: in higher plants, unicellular organisms, fungi, vertebrates and invertebrates. The development of cytological techniques made it possible to observe in more detail the cellular damage, as opposed to the response of the whole organism or tissue, which is brought about by ionizing radiations. Thus chromosome and chromatid breaks were found in irradiated cells. The delayed death of the cell and certain types of mutations could be attributed to these nuclear effects.

---

\* Translated by SPEAR<sup>2</sup> as follows: 'The sensitivity of cells to irradiation is in direct proportion to their reproductive activity and inversely proportional to their degree of differentiation'.

This chapter is devoted to a summary of these events; we shall attempt to use the precise terminology of cyto-genetics while still making the treatment comprehensible to the non-specialist. The geneticists (like some embryologists) have developed by degrees a specialized language which makes the reading of the literature extremely difficult for those with a limited biological training. This extreme specialization also tends to isolate the geneticist and leads to abstract speculation concerning complex phenomena which distract attention from the underlying physicochemical processes. The cytologists have themselves become aware of this and DARLINGTON<sup>3</sup> writes: 'Chromosomes studies have long promised to become a kind of visible chemistry. This promise is now being realized'; he further indicates how geneticists can benefit from cellular physical chemistry. The integration of biochemistry and physiology with cytology and genetics remains a task of almost insurmountable difficulty; but a start may be made by phrasing the problems in such a way that the different disciplines can contribute to their solution. Obviously a fundamental requirement is that the experimental techniques used should be reliable.

Cytologists are becoming more and more conscious of the fact that artefacts are introduced by the drastic methods used in the classical fixation procedures. Those methods which gave the sharpest definition and the best pictures are now known, largely as the result of the work of CLAUDE<sup>4</sup> and DANIELLI<sup>5</sup> to disturb the structure of the cell and the localization of its various constituents. Contemporary cytologists are adopting 'physiological' methods such as tissue culture, phase-contrast microscopy and cinematography which make it possible to study the *living* cell.

#### MITOSIS

It is proposed to describe cell division, mitosis, in the simplest terms, and to see how these processes are modified by irradiation (see *Figure 1*).

In general the nucleus is separated from the cytoplasm of a 'resting' cell by a membrane. Little is known concerning its physical properties except that it must be more permeable than the cell membrane as exchange between the cytoplasm and the nucleus occurs freely. In the resting nucleus only a few structures can be recognized under the microscope; most notable is the nucleolus which does not appear to contain desoxyribosenucleic acid (DNA) but like the cytoplasm contains ribosenucleic acid (RNA).

## CYTOLOGICAL AND GENETICAL PHENOMENA

At the start of mitosis during the period known as *prophase*, dense filaments capable of being stained with basic dyes appear in the nucleus. As division proceeds these filaments become shorter and form spirals. On favourable material one can see that they are composed of two threads, known as chromatids, which may be revealed by special techniques as in *Figure 1*. The stage at which the chromosomes divide or 'split' into two chromatids has not been unambiguously determined and there has been considerable controversy between workers using different materials. At present one has to be satisfied with the statement that in prophase the chromosomes are already double. Next, the nuclear membrane disappears and the chromosomes become free in the cytoplasm. At this stage, called *metaphase*, a spindle (see *Figure 5*) becomes apparent in the cytoplasm. It is believed to be composed of fibrous proteins, which are in a 'liquid-crystalline' state. The polypeptide chains are thought to run parallel to the axis of the spindle and to be perpendicular to the equatorial plate in the centre of the cell where the chromosomes assemble. The spindle extends to the opposite poles of the cell and each chromosome attaches itself to a fibre of the spindle at a single point, the centromere, which can be identified as the chromosomes are usually bent at this juncture. In certain specialized cells the centromeres of the chromosomes fuse into a large chromocentre (see *Figure 3*). During prophase the chromosome does not split at the centromeres; it remains single until the end of metaphase. At metaphase one cannot observe distinctly that the chromosomes are composed of two identical threads because they are highly contracted and the whole chromosome appears single.

In the next stage of the mitotic cycle, *anaphase*, the chromosomes divide; the chromatids separate in the region of the centromeres where they had remained undivided or single up to the end of metaphase. The onset of anaphase is determined by chromosome splitting at this point. This separation is extremely rapid—almost explosive—and each of the two chromatids—now known as daughter chromosomes since they are independent threads—move towards the opposite poles along the spindle. This process insures that chromosome material is exactly halved between the two daughter cells.

During *telophase* the chromosomes become gradually less dense (*i.e.* they swell), elongate and fuse into a fine network of chromatin which eventually becomes invisible. The nuclear membrane is reformed, the nucleus becomes spherical and nucleoli appear. The presence of a distinct nucleus is characteristic of the resting stage and the cell enters *interkinesis* or *interphase*. The cytoplasm is

## MITOSIS

divided by a new cellular membrane which completes the formation of the two daughter cells.

The key problem confronting the cytologist is the mechanism by which chromosomes disappear at the end of telophase and reappear duplicated with exactly the same morphological and genetical organization in the subsequent prophase or metaphase. Geneticists postulate—and upon this postulate current concepts of genetics rest—that the chromosomes preserve their structure during interphase. Although they are invisible each chromosome filament divides into two or reproduces itself. In the subsequent mitosis the chromosome again becomes visible under the microscope and is composed of two identical daughter chromatids.

Classical workers have not paid much attention to what occurs in the cytoplasm during the 'chromosomal ballet'. It is usually stated that the cytoplasmic mass, which grows during the inter-phase, is divided into two approximately equal parts. The constriction imposed by the membrane, which is formed between the two separate nuclei, divides the daughter cells. In the cytoplasm there are well-defined bodies which are, however, less rigidly fixed in space and very much more persistent than the chromosomes. These structures vary in size; those visible in the optical microscope are known as mitochondria, and bodies smaller than these as microsomes. It is assumed that these cytoplasmic bodies have the power of reproduction and they have been compared by HADDO<sup>7</sup> with viruses. These organs contain many enzymes and are rich in phosphatides and ribonucleic acid (RNA); they are surrounded by an 'imperfect' membrane and are sensitive to changes in osmotic pressure (*e.g.* salt concentration) in the outside medium.

In the absence of evidence to the contrary, one assumes that the two daughter cells besides having the same number of chromosomes also possess approximately the same cytoplasmic equipment. Phase-contrast cinematography of mitosis in tissue cultures reveals that the cytoplasm as well as the nuclei undergoes rapid changes. The formation of a number of cytoplasmic bubbles which at first swell and then shrink again can be observed on the surface of the cell membrane in tissue culture indicating profound biochemical or physiological change.

## MEIOSIS

The growth of an isolated tissue is nearly always the result of cell multiplication by cell division. The reproduction of complete multicellular organisms is more often confined to special cells, the

#### CYTOLOGICAL AND GENETICAL PHENOMENA

gametes (sperm-ovum). A study of these special cells revealed that growth and heredity are complementary\*.

The division which precedes the formation of gametes (sperm and ovum), meiosis, differs in some respects from mitosis. In mitosis the chromosomes are divided equally, i.e. at the end of the division each daughter cell has the same number of chromosomes as the parent cell. The gamete on the other hand carries only half the number of chromosomes each and the original number is re-established by their fusion. This process occurs at fertilization.

The fertile egg, the first diploid cell, has  $2n$  chromosomes of the new organism and each pair of chromosomes is composed of one maternal and one paternal chromosome (and its genes). Based on this observation the recombination of parental characteristics and the permanence of chromosome numbers in the offspring can be understood. Meiosis consists of two successive divisions frequently without an interphase. The nuclear network of chromatin resolves into long threads which represent *single* chromosomes. The homologous chromosome threads then pair side by side, and this is followed by division of each paired chromosome into two sister chromatids. At the same time at certain loci the chromatids of homologous chromosomes break and exchange partners†. This exchange may be observed cytologically and is known as chiasmata (see Figures 4 and 7).

The paired chromosomes contract and after the disappearance of the nuclear membrane they form a characteristic configuration (called the bivalent) on the equatorial plate in which each member is oriented and moves to the opposite poles undivided. As a result the two daughter cells have only half the number of chromosomes of the mother cell. Without a resting stage the chromatids of each chromosome separate and undergo an ordinary process of mitosis. In this way a group of four cells is produced each of which becomes a pollen grain or a mature sperm.

The same processes take place during the formation of the female

\* DARLINGTON and MATHER<sup>6</sup> write '... we find that the centre of the control of development is the nucleus whose sphere of action is the cell. The nucleus, by way of the cell, controls both heredity and development. In development, the nucleus provides the constant basis for the regular changes in the cytoplasm, changes which could not be regular unless they had that constant basis. The nucleus and the cytoplasm have long seemed to be the opposite poles of biological study; but their new unity need no longer surprise us. Heredity now appears as the repetition of the series of changes which constitutes development, and the study of each is bound to require more and more the understanding of the other'.

† This process is called 'crossing-over' and is the most important event in the life of a chromosome as it is the mechanism by which chromosomes can qualitatively vary their genetic composition or content.

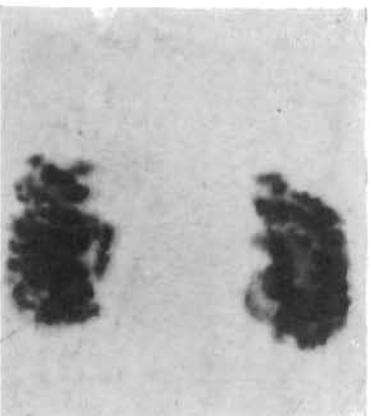
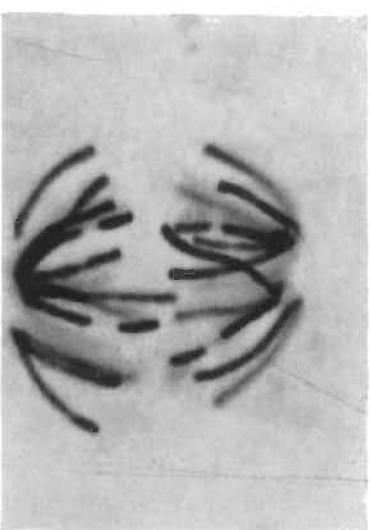
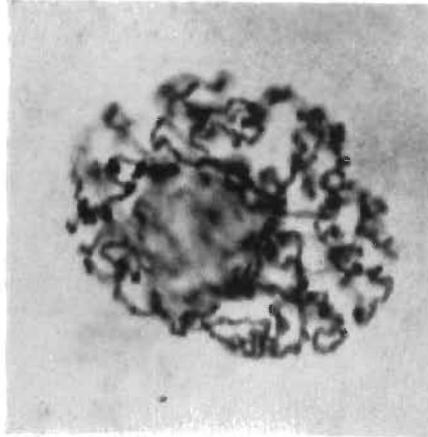


Figure 1. Mitotic cycle of cells from *Vicia faba*. (a) Early prophase; (b) Late prophase; (c) Metaphase; (d) Metaphase (side view); (e) Anaphase; (f) Telophase

To face p. 160

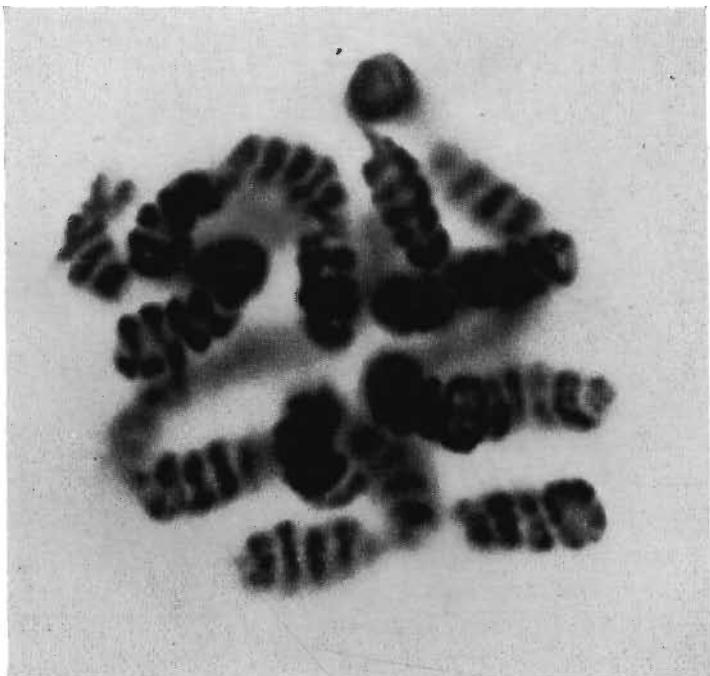


Figure 2. Spiral structure of chromosomes in *Tradescantia virginiana* (revealed by pretreatment with nitric acid vapour). Magnification  $\times 2400$



Figure 3. Salivary gland chromosomes of *Drosophila* showing banded structure and fusion of the chromosomes into the chromocentre. Magnification  $\times 600$

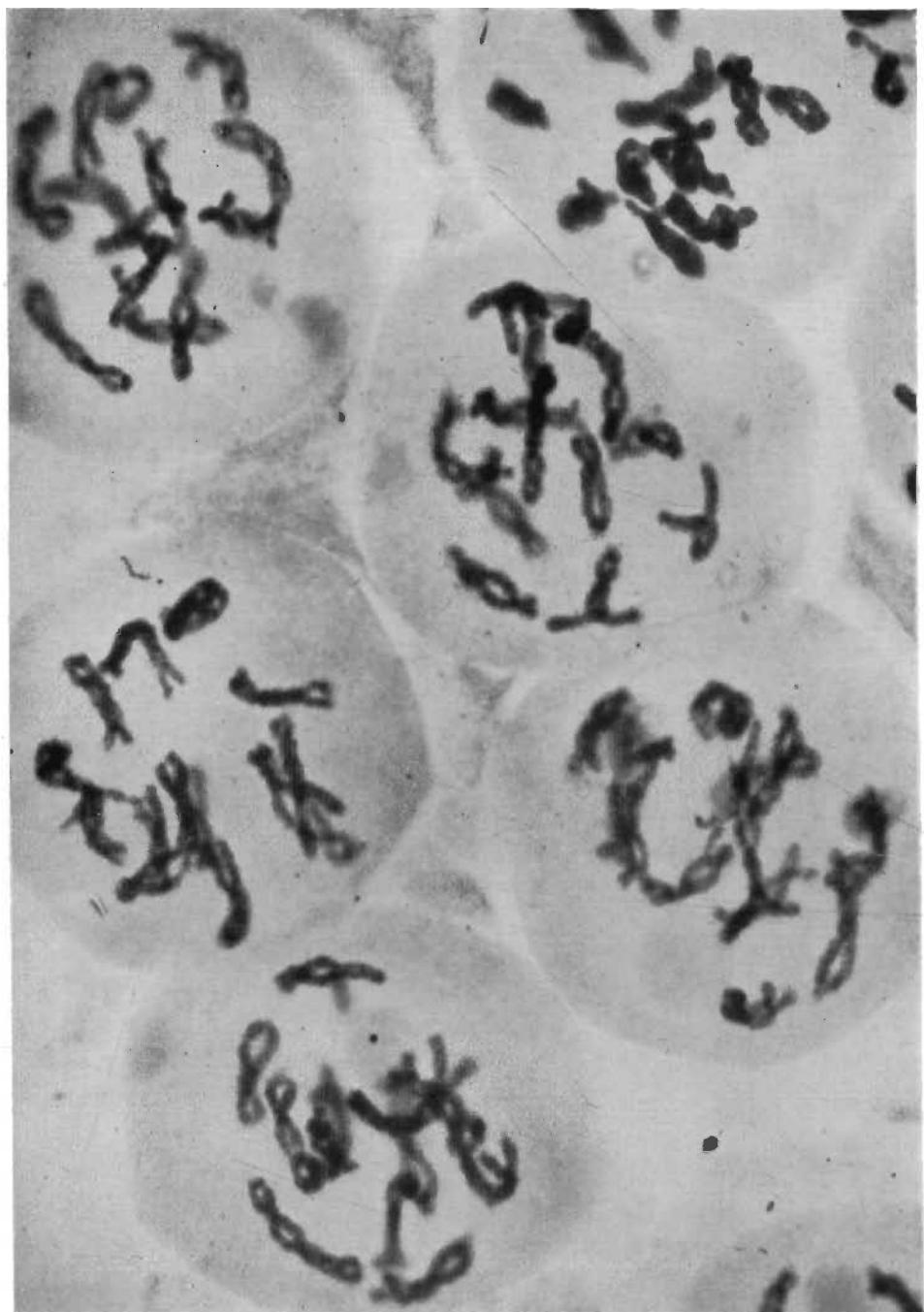


Figure 4. Pollen mother cells in *Fritillaria pallidiflora* showing chiasmata where exchange of sister chromatids has occurred. Magnification  $\times 1500$



Figure 5. Dividing cell in fish embryo showing the mitotic spindle. Magnification  $\times 1500$



Figure 6. Physiological effect of radiations (stickiness) in a rat tumour (Walker carcinoma) 6 h after a dose of 100 r of x-rays. Magnification  $\times 4000$

To face p. 161

## MEIOSIS

gamete (egg or ovum) except that the mother cell (or oocyte) is generally very large and well supplied with food reserves. The meiotic divisions affect only the nuclear material and are near the membrane so that at the end of the meiosis one large haploid cell

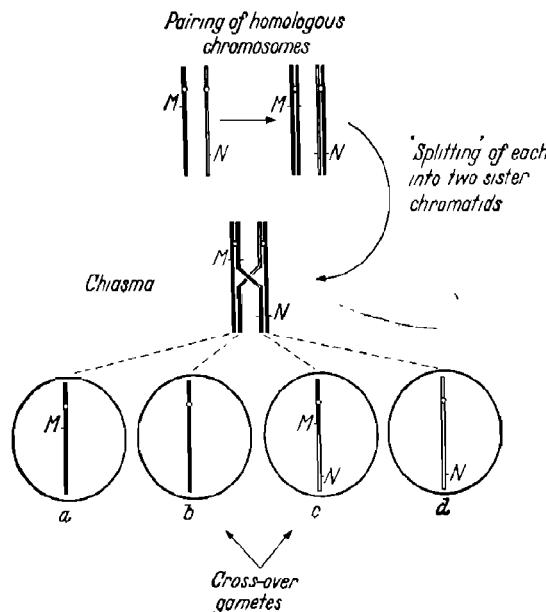


Figure 7. Diagram to illustrate the process of crossing-over. The four different gametes are obtained from one crossing-over and shows how two genes (M and N) in different homologous chromosomes will be distributed within the gametes.<sup>8</sup>

remains which becomes the egg while the three small nuclear masses, called the 'polar bodies' rapidly degenerate.

### VISIBLE CHANGES PRODUCED BY IONIZING RADIATION

The materials most widely used by cytologists in these investigations are pollen grains (in particular certain varieties of *Tradescantia*), the growing roots of the broad bean (*Vicia faba*), the onion (*Allium cepa*), and various species of hyacinth, *Fritillaria* or *Trillium*. All these possess the advantages of having large chromosomes and frequent and regular mitoses. Sparrow found that *Trillium erectum*, a wild flower common in the north-east of the United States, has five pairs of chromosomes and its large anthers contain many pollen mother-cells which divide simultaneously, i.e. all the pollen matter in the cells is in the same stage of division.

## CYTOTOLOGICAL AND GENETICAL PHENOMENA

*Permanent and temporary effects*--KOLLER<sup>8</sup> groups the changes observed in dividing pollen grains of *Tradescantia bracteata* after very low intensity irradiation, as follows:

- (a) Stickiness of chromosomes
- (b) Breakage of the centromere region
- (c) Errors in the formation of the spindle
- (d) Errors in spiralization (spurious breakage)
- (e) Chromatid (B') and chromosome (B'') breaks
- (f) Formation of nuclear fragments (micro-nuclei).

The first four are called physiological or temporary effects (see Figure 6) as they are manifested almost immediately and last only for a few hours after irradiation. They do not lead to any permanent modification of the chromosomes (*i.e.* mutations) but can occasionally cause the death of the cell.

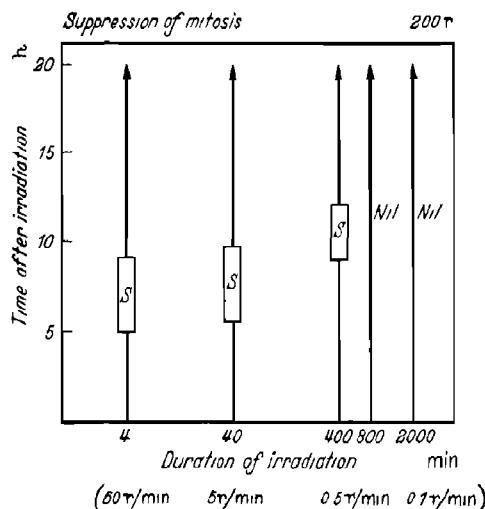
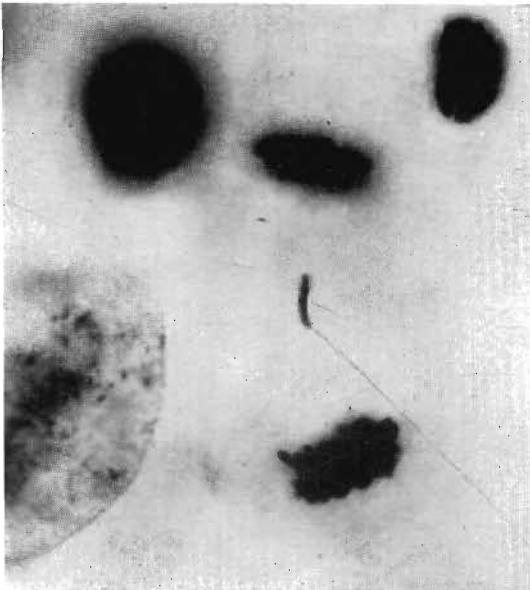


Figure 8. Diagram to illustrate the influence of dose rate on the suppression of mitosis in *Tradescantia* pollen grains produced by 200 r of x-rays (—S— represents period of suppression of mitosis)<sup>8</sup>

To produce the 'physiological effects' the dose rate is of great importance. For example, no stickiness is observed if *Tradescantia* pollen grains are exposed to 50 r of x-rays at 0.1 r/min while the same dose given at 0.5 r/min produces stickiness in half the cells which are undergoing division. A possible interpretation is that the radiation\* induced biochemical changes which cause this

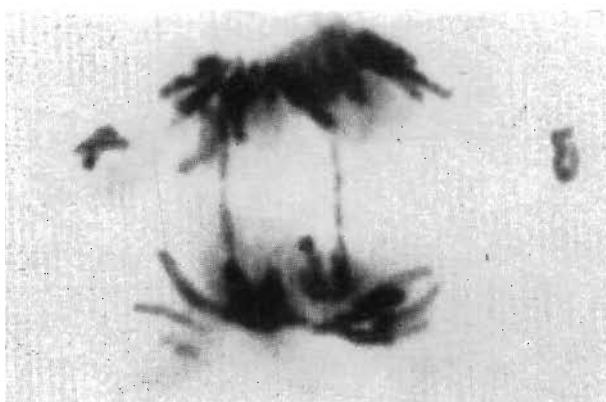
\* LEA<sup>9</sup> assumes that the 'physiological effects' are due to energy absorption of the radiation in the cytoplasm, in contrast to the genetical or permanent effects which are due to nuclear action. If Lea's interpretation is correct then this is an example of *action at a distance* (*i.e.* a biological lesion in one part of the organism produces a response in another part). *Action at a distance* must not be confused with *indirect action* (see Chapter 2).

25 r



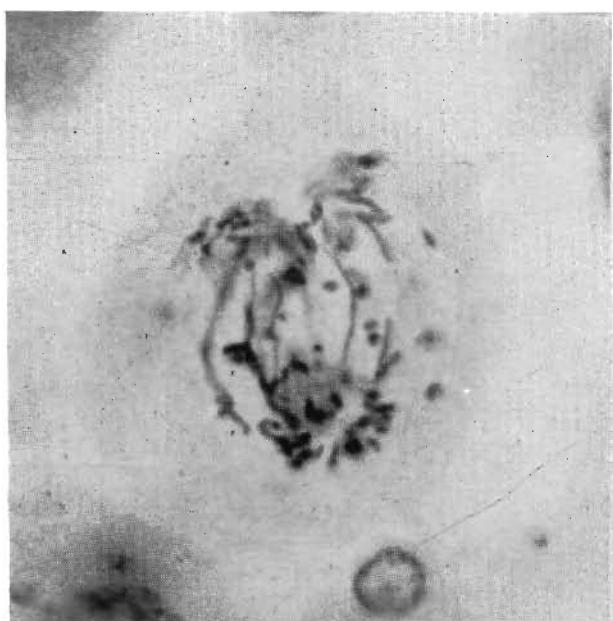
a

300 r



b

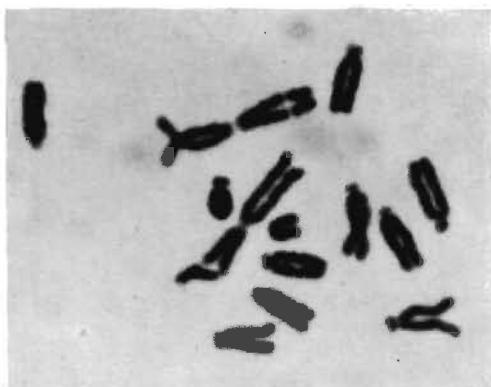
1000 r



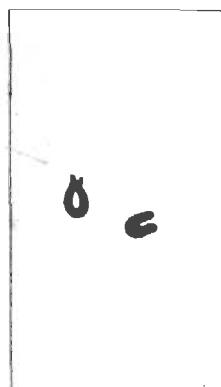
c

Figure 9. Chromosome abnormalities in rat tumour cells (Walker carcinoma) visible at mitosis after different doses of x-rays

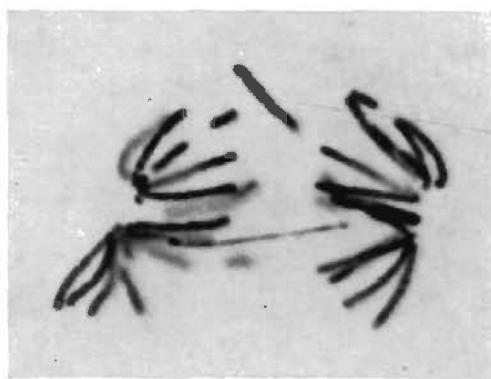
To face p. 162



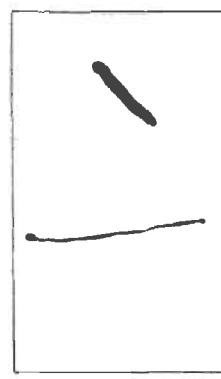
a



a



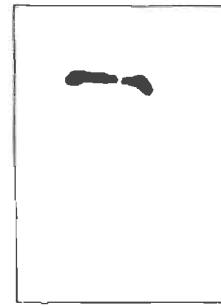
b



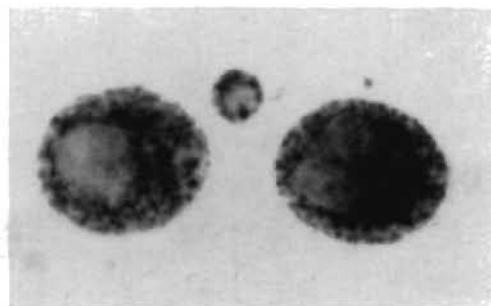
b



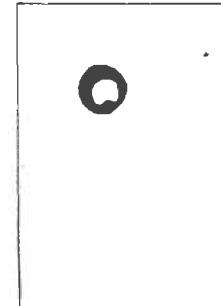
c



c



d



d

Figure 10. Alteration in the mitotic cycle of *Vicia faba* after exposure to X-rays (compare with Figure 1). (a) Metaphase with one chromosome break. (b) Anaphase with 'bridge and fragment' derived from a chromosome break. (c) Telephase with fragment excluded. (d) Resting stage with micronucleus

#### VISIBLE CHANGES PRODUCED BY IONIZING RADIATION

stickiness do not accumulate with dose at low dose rates. At high intensities the disturbances produced are not repaired and as a result stickiness appears.

To produce a detectable change in the spindle of *Tradescantia* a relatively high dose (150 r) must be given but the disturbance only becomes apparent about seven hours later. The arrest of mitosis, which the classical radiobiologists consider to be one of the most characteristic radiobiological effects, is only produced in the pollen grains of *Tradescantia* if 200 r of x-rays are given at a rate of not less than 0.5 r/min. The inhibition lasts about four hours (see *Figure 8*). Quite unexpected was the observation that irradiation at the very low rate of 0.1 r/min increases the frequency of mitosis from 38 to 67 per cent. There can be no doubt that this is a genuine increase in the number of cells which enter prophase and not a delay or slowing down of metaphase or anaphase.

*Breakage of chromosomes and chromatids*.—The phenomenon (see *Figures 9–10*) on which the attention of cyto-geneticists is concentrated is the fragmentation of chromosomes and the subsequent events. Some changes produced in chromosomes can be reproduced in the divisions following treatment and may therefore become perpetuated. Since chromosomes carry the genes (*i.e.* the molecular complexes which control the morphology, development and behaviour of organisms) permanent structural changes may result in genetical changes.

The analysis of the radiation effects on chromosomes of various plant and animal material led cytologists to the assumption that breakage can occur either before or after the longitudinal division of the chromosomes into two chromatids (*i.e.* before prophase) has taken place. According to the symbols proposed by DARLINGTON and LA COUR<sup>10</sup> the chromosome break is written B''. The breakage of chromatids, symbolized by B', occurs after the division of the chromosomes (see *Figure 11*). The cytological observations are usually made in metaphase when a very modified chromosome structure is seen from which the initial event (B' or B'') is deduced and every 'observation' contains therefore an element of interpretation\*. As an example we may mention the difference in interpretation concerning the origin of chromatid breaks: (a) breakage occurs after the chromosomes have 'split' and is restricted to one chromatid only; (b) breakage occurs in the single chromosome before its division into two chromatids, and after the 'split' the break

\* In this chapter the radiation effects on chromosomes will be interpreted on classical lines as 'breaks'. REVELL<sup>20</sup> has recently proposed an entirely different mechanism which has many attractive features.

## CYTOLOGICAL AND GENETICAL PHENOMENA

in one chromatid undergoes restitution while the other remains broken.

As the structural changes induced by radiation often occur during the resting stage or interphase and do not become visible before the end of the following prophase, it is assumed by many cytologists that

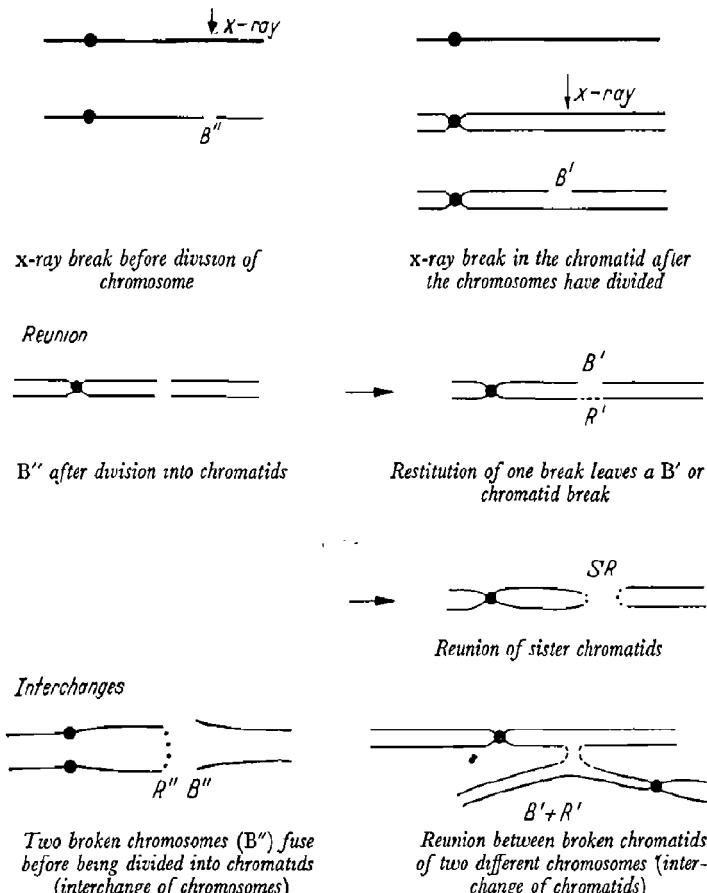


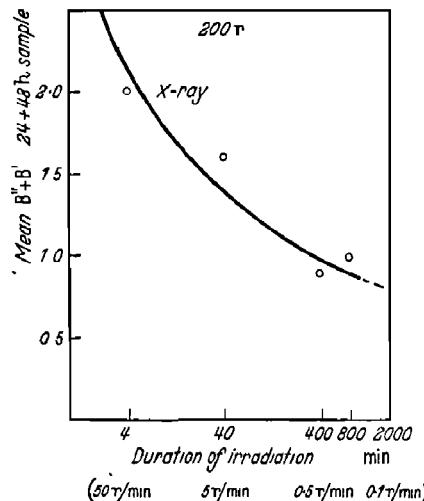
Figure 11. Diagram to illustrate breakage of chromosomes (B'') and of chromatids (B') and the subsequent events

a high proportion of the breaks are restituted (*i.e.* undergo complete fusion in the original way). If it is accepted that this phenomenon is frequent the number of breaks counted—*i.e.* those the observer can see as open breaks or infer from reunions—depends on the size of the gap and on the interpretation of the interchanges rather than on the frequency of the initial lesions induced. These quantitative

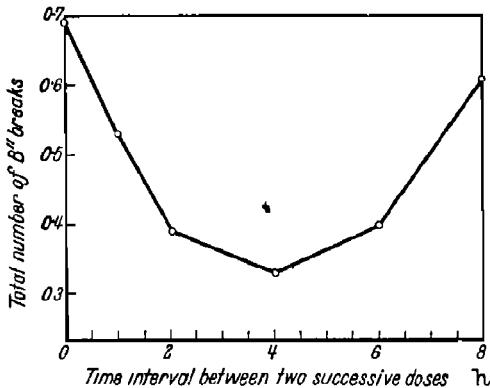
### VISIBLE CHANGES PRODUCED BY IONIZING RADIATION

treatments based on the *observed* number of breaks are clearly of very limited value.

When the irradiation is given at low intensity so as to induce chromatids ( $B'$ ) and chromosome ( $B''$ ) breaks, the duration of the irradiation is more important than the total dose. The total number of  $B' + B''$  increases with increasing dose rate (see *Figure 12*).



*Figure 12.* Relationship between total number of breaks ( $B' + B''$ ) observed in *Tradescantia* pollen and the dose rate at which 200 r of x-rays were delivered<sup>8</sup>



*Figure 13.* The influence of the time-interval between two successive doses of x-rays on the total number of chromosome ( $B''$ ) breaks observed in *Tradescantia* pollen<sup>12</sup>

According to KOLLER<sup>8</sup> the reduction in the number of breaks with decrease in dose rate cannot be attributed to increased restitution alone as postulated by SAX<sup>11</sup> and other factors must contribute to this effect.

The possibility that irradiation modifies the radiosensitivity of chromosomes was indicated by LANE<sup>12</sup> in experiments in which the x-ray dose was fractionated (see *Figure 13*) and the interval varied.

### CYTOTOLOGICAL AND GENETICAL PHENOMENA

between successive short exposures at moderate dose rates. If an interval of four hours is interposed the number of abnormalities recorded for *Tradescantia* was reduced to about one-half. When the period was extended to eight hours the breakage frequency recovered to 85 per cent of that observed with a continuous dose. According to Lane the effect of fractionation with long time-intervals on the

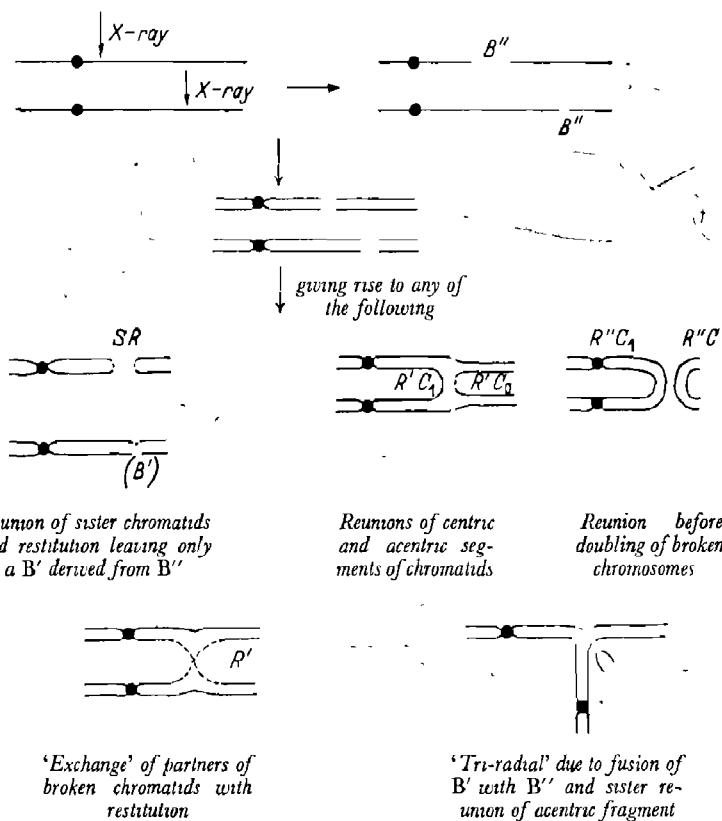


Figure 14 Diagram to illustrate some of the different types of reunions between two broken chromosomes

number of breaks produced cannot be due to any change in restitution and indicates that irradiation brings about a temporary change in sensitivity of the chromosome to breakage. This important interpretation needs further verification. Lane's observations also throw light on the hypothesis used in this discussion that restitution and rearrangement of the broken chromosomes do not take place immediately.

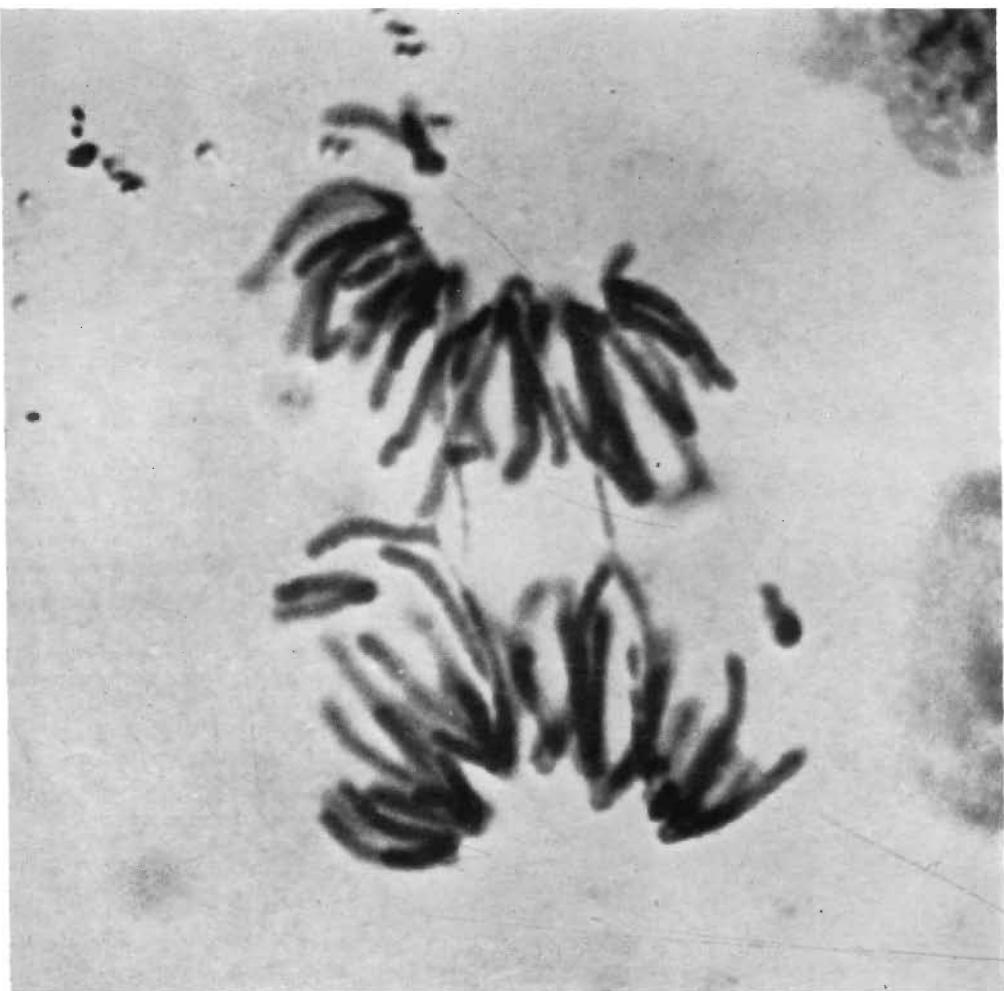


Figure 15. Bridges and acentric fragments in a dividing cell in the tail of the tadpole (Triton) 48 h after 125 r of x-rays



Figure 16. Representative chromosome abnormalities produced in *Vicia faba* by x-rays

- (a) Normal mitosis
- (b) Chromatid exchange
- (c) Chromatid isochromatid exchange
- (d) Chromatid break and chromosome exchange

#### VISIBLE CHANGES PRODUCED BY IONIZING RADIATION

Chromosomes broken by ionizing radiation can behave in essentially three ways (see *Figures 14-16*): (i) The broken chromosome may reunite (repair) in the original way; (ii) the chromosome may remain in a fragmentary state; or (iii) the fragments may reunite in new combinations\*; fusion can take place between sister chromatids or between non-homologous chromatids and chromosomes. When for example in a diploid cell two chromosomes are broken before the 'split' in prophase there will be four broken chromatids available for recombination, leading to a large variety of possible new configurations.

The broken chromatids from the same chromosome (sister chromatids) often reunite and the process is known as sister reunion (SR). In this case one of the pairs formed will be without a centromere (termed an acentric fragment). Such fragments are inert and are left in the cytoplasm because having no centromere they cannot move on the spindle. The two chromatids from the same chromosome may fuse with two chromatids of a homologous chromosome to give a configuration like an X. Tri-radial configurations, translocations, inversion, duplications, deficiencies and dicentric chromosomes can also be seen.

The chromosome bridges, which are formed by dicentric chromosomes and which survive until the end of mitosis, usually join two chromosome groups together. These abnormalities are not important genetically because both daughter cells degenerate and the process is not perpetuated. However, if these bridges break and each daughter cell receives an almost equal amount of chromosome material, then during the resting stage new dicentric chromosomes may be formed by sister reunion after the reproduction of the broken chromosome (see *Figure 17*). The two centromeres of the same chromosome during the following mitosis can move towards the opposite poles of the spindle, the chromosome bridge breaks and a new cycle of break-fusion-bridge starts again. The fact that such a cycle has been found to persist through thousands of mitosis (*e.g.* in a certain variety of hyacinth) emphasizes the value of the purely structural interpretation given to chromosome breaks by cytogeneticists. All these rearrangements are of the greatest importance from the genetical point of view, since these structural changes make it possible for the chromosome material to divide unequally giving daughter cells which differ in their genetical composition.

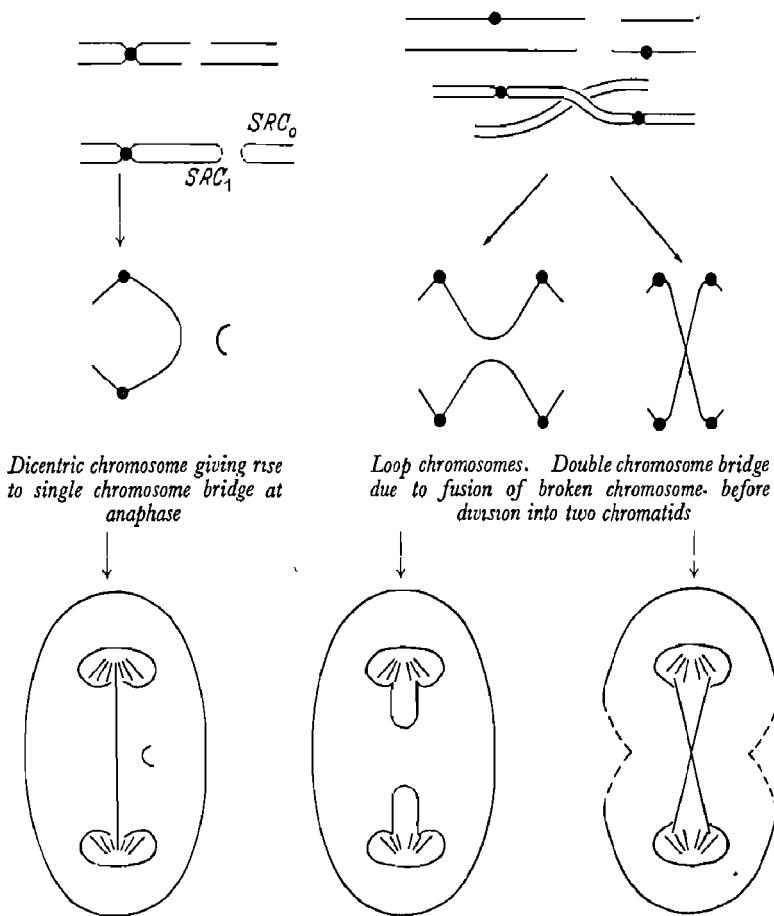
It has already been mentioned that the total number of breaks increases with increasing dose rate. The relative frequency of the

---

\* Breaks produced in the chromosome of maize and *Tradescantia* by u.v. are permanent and do not undergo fusion or reunion.

## CYTOLOGICAL AND GENETICAL PHENOMENA

different rearrangements of the broken chromosomes also varies with dose rate, e.g. the proportion of reunions of sister chromatids is greater in cells in which the chromosomes have suffered a smaller number of breaks. The breaks are usually distributed randomly along the chromosome (see *Figure 7* on p. 200) although some examples are known in which certain loci appear to be 'weak' and



*Figure 17. Diagram to illustrate origin of anaphase bridges*

break more frequently than would be expected by chance. Though in recent years many detailed examinations have been carried into the breakage frequency of different chromosomes and into the different types of reunions and their structural consequences, it is questionable whether this preoccupation with the mechanics of chromosome breaks has increased our understanding of the fundamental mechanisms involved.

#### VISIBLE CHANGES PRODUCED BY IONIZING RADIATION

Many cytologists believe that the chromosome breaks induced by ionizing radiation are largely responsible for the delayed death of the affected cells and the cause for certain genetical effects. In neither case, however, is the relationship direct, thus dose rate while influencing the frequency of observed abnormalities does not affect the frequency of gene mutations. Also, the RBE (see p. 58) for different radiations is not the same for the killing of cells and the production of chromosome breaks (see p. 75). D'AMATO and GUSTAFSSON<sup>14</sup> observed that the frequency of chromosome interchanges in barley after irradiation is increased by colchicine, uranyl nitrate, hydrogen peroxide and ferric sulphate, all substances which are chemical mutagens for the material used. On the other hand, relatively concentrated (0.01M) potassium cyanide decreases the mutagenic effect of x-rays while increasing the frequency of chromosome aberrations. (Note also that cyanide is a good radiation protective agent, see Chapter 14). D'Amato and Gustafsson summarize their conclusions as follows:

'This result is of profound interest since it indicates that an increase of chromosome aberrations—translocations, inversions, deficiencies—does not necessarily raise the amount of visible mutants. It argues against the view that visible mutations are nothing but chromosome rearrangements, or at least, that they are always connected with the origin of chromosome disturbances. In this special case, we conclude that concentrated solutions of potassium cyanide increase the rates of chromosome breakage, although the genic material does not react with an increase of visible mutants'.

Some of the facts which are related to chromosome breakage and which must be borne in mind when considering the effects of radiation may be summarized briefly thus:

(i) Many chemical substances—referred to as mutagens—are now known which induce both gene-mutations and chromosome breaks (see Chapter 7).

(ii) Breaks in chromosomes are observed in the absence of chemical or physical agents (*e.g.* in *Tulipa fragrans* the frequency of spontaneous\* or naturally occurring breakage is very high).

(iii) When the chromosomes have been greatly affected, cellular division becomes so abnormal that after one or two cell generations further divisions become impossible and the cell dies.

---

\* The term spontaneous is used because the underlying chemical mechanism is not known. The recent observation of CONGER and FAIRCHILD<sup>13</sup> that an increase in the amount of oxygen in the atmosphere increases the frequency of chromosome breaks in certain pollen grains may point the way to the understanding of spontaneous breaks.

## CYTOLOGICAL AND GENETICAL PHENOMENA

(iv) Ionizing radiation and many chemical mutagens are also carcinogenic.

### THE ROLE OF THE CYTOPLASM

Two interesting experiments show that nuclei isolated from the cytoplasm are very radioresistant. EULER and HAHN<sup>15</sup> isolated the nuclei from calf thymus cells and irradiated them with high doses of x-rays ranging from 25,000 to 60,000 r. The nucleoprotein extracted from these cells could not be distinguished in its physical and chemical properties from that obtained from unirradiated cells.

DURYEE<sup>16</sup> carried out a remarkable investigation with nuclei or eggs taken directly from the ovary of frogs and salamanders. The nuclei freed of cytoplasm by microdissection are very radioresistant and after 30,000 r no lesion can be seen; only after 60,000 r can change in the nucleolus be observed. The nucleus recovers its radiosensitivity if it is replaced in the cytoplasm and on irradiation the reconstituted cell shows all the typical radio lesions except breakage of chromosome filaments.

If as little as  $10^{-4}$  ml of the cytoplasm of a cell, irradiated several days previously, is injected close to the nucleus with a micropipette into the cytoplasm of a non-irradiated cell, the latter shows, one to two hours after the injection, all the symptoms of radio lesions such as rupture of the nucleolus, pycnosis, vacuolization and chromosome fragmentation. Out of a total of 26 such experiments 23 gave a positive result; while in a control series where unirradiated cytoplasm was injected into normal cells, nuclear abnormalities were observed only in 5 out of 35 experiments.

On the basis of these and other experiments, Duryee has formulated the following sequence of events for the production of radio lesions and his theory agrees perfectly with the concept of a biochemical mechanism as opposed to a mechanical interpretation for the nuclear effects of radiations.

First, production of localized chemical events which are temperature insensitive, and occur instantaneously at the molecular level; second, accumulation of toxic catabolic substances—highly temperature sensitive; third, transmission of toxic products and accumulations in various parts of the cell including the nucleus.

Another (and more physiological) technique for studying if irradiation of the cytoplasm can influence non-irradiated chromosomes consists of irradiating female insects, *Drosophila* and *Habrobracon*, and mating them immediately with a non-irradiated male. Unlike the experiments of Duryee the results were wholly negative.

#### THE ROLE OF THE CYTOPLASM

In the case of the wasp the irradiated cytoplasm killed the embryo but failed to act on the intact nuclear material brought by the spermatozoa. Yet another method of studying the same problem was developed by ZIRKLE and BLOOM<sup>17</sup>. They irradiated cells from the heart of newts in tissue culture with very fine ( $2.5\mu$ ) beams of protons and were thus able to localize the action of the radiations. After a few protons had passed through part of the chromosomes typical cytological effects such as breakage were observed. Even after the passage of many thousands of protons through the cytoplasm immediately adjacent to the chromosomes no visible abnormalities were produced. It may be that the use of the densely ionizing protons is responsible for the results obtained by Zirkle and Bloom differing from those of Duryee. Another possibility is that in some cases the cytoplasmic proteins can act as protective agents. Such a conclusion may be justified from the experiments of BACK and BLOCH-FRANKENTHAL<sup>18</sup> who showed that the oxygen consumption of isolated nuclei from the blood corpuscles of the chicken was influenced much more by irradiation with x-rays *in vitro* than by irradiation of the whole blood.

#### MECHANISM OF CHROMOSOME BREAKAGE

Unfortunately it is not possible to end this brief survey with any definite conclusion. In the authors' view experiments with substances which offer protection against some of the effects of ionizing radiation (see Chapter 14) may throw much light and possibly serve to reduce the complexity of the cytological and genetical studies.

We have seen in Chapter 2 that the purely mechanical interpretation of chromosome breakage can no longer be maintained. Clearly the impact of an ionizing particle with a chromosome cannot be compared with the snapping of a telephone wire by a bullet. This is very clearly brought out by GRAY<sup>19</sup> who showed that chromosome damage occurred in every cell of *Tradescantia* which had been traversed by one  $\alpha$ -particle (see *Figure 18*), although the probability of one  $\alpha$ -particle hitting a chromosome is very small. The mathematical analyses which have been developed on the direct-hit theory are seen to be fortuitous, and this is not surprising in view of the large number of arbitrary assumptions which had to be made to fit the data. The fact that the sensitivity of the chromosomes to breakage by x-rays can be modified both by prior exposure to ionizing radiation (see p. 166) and by exposure before or after irradiation to infrared (see p. 201) also emphasizes that chromosome injuries are not the direct result of the passage of an ionizing particle but are the

## CYTOLOGICAL AND GENETICAL PHENOMENA

result of a complex interplay of different factors in a living system which reacts to changes brought about in its environment.

One must, however, be cautious in abandoning entirely some of the concepts of the mechanical theory of chromosome breakage in favour of a purely biochemical mechanism based on indirect action. The great complexity of the response of the cell to radiation probably permits the simultaneous occurrence of a number of quite different processes, and a synthesis of the different views may be necessary.

Since we know that many chemicals (see Chapter 7) can provoke radio lesions indistinguishable from those produced by radiation—

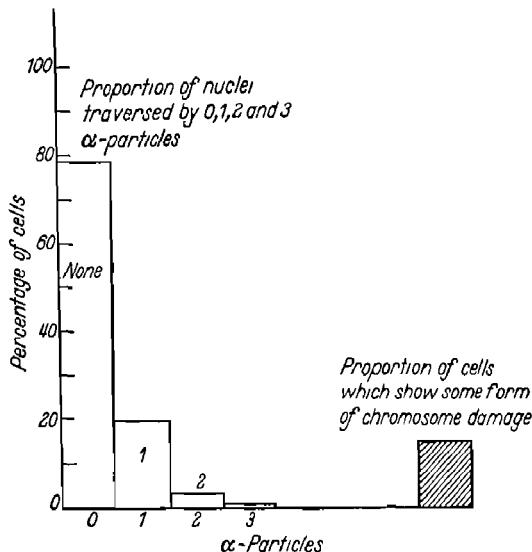


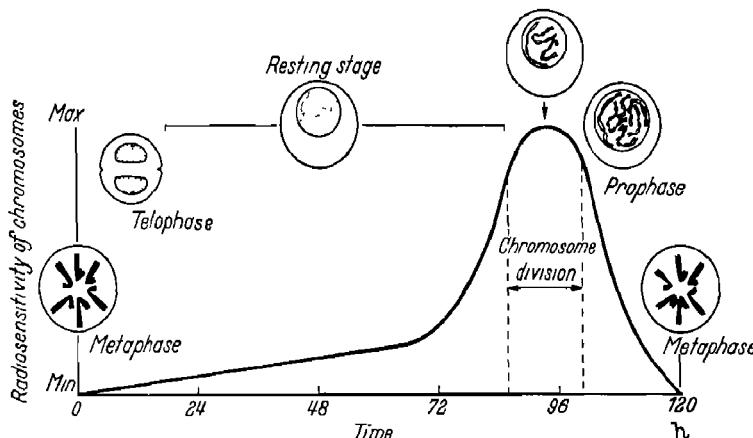
Figure 18. Relationship between proportion of *Tradescantia* pollen traversed by 0, 1, 2 and 3  $\alpha$ -particles respectively and the percentage of cells showing some form of chromosome damage<sup>19</sup> following an exposure to 4 rep

although differences in mechanism are now becoming apparent—and as the action of radiation in the cell is almost certainly indirect (*i.e.* produced by the highly reactive chemical substances known as free radicals) it is tempting to consider chromosome breakage purely as a physiological response to a biochemical disturbance. This may be true for lesions which require a certain time before they become apparent. How far this is the case for chromosome breakage is not yet certain. We have already stated that the breaks in the chromosomes only become visible at the end of prophase, although it has been established that the greatest sensitivity in the life of the nucleus is at the end of the resting stage (see *Figure 19*). This period co-

### MECHANISM OF CHROMOSOME BREAKAGE

incides with the stage where geneticists on various evidence assume that the doubling or splitting of the chromosome occurs. As there is little breakage at prophase, chromosomes are clearly not cut by radiation like grass with a scythe. On the other hand, the end of the resting stage is not a period where DNA synthesis occurs as the threads are believed to be already formed. The chromosome breaking chemicals which probably interfere with synthesis act at a much earlier point in the resting stage.

Another factor which we have already stressed (see pp. 71 and 169) and which argues strongly against a purely biochemical mechanism (such as interference with synthesis) is that chromosome breakage is produced much more readily by radiation of high



*Figure 19. Diagram to show the sensitivity of chromosomes to radiation during the developmental cycle of Tradescantia pollen grains. 200 r of x-rays were given at different times and the sensitivity measured by the frequency of chromosome breaks observed at metaphase<sup>8</sup>*

specific ionization. Thus, a dose of 5 MeV  $\alpha$ -particle may, under favourable conditions, be 10 to 20 times as effective as a similar amount of energy delivered by x-rays. This proves clearly that chromosome breaks are not produced by inactivation of enzymes or alteration of isolated macromolecules as radiation of low specific ionization should then be most effective. The high local concentration of free radicals produced by densely ionizing radiation would be particularly effective in disrupting an organized structure or pattern of macromolecules. A process of this kind occurring at the end of the resting stage would appear to be very effective in producing a chromosome breakage. It is possible that interference by ionizing radiation with enzymatic processes connected with synthesis also results in the appearance of breaks, though with a much lower efficiency.

CYTOTOLOGICAL AND GENETICAL PHENOMENA

REFERENCES

- <sup>1</sup> BERGONIÉ, J. and TRIBONDEAU, L., *C.R. Acad. Sci., Paris*, 1906, **143**, 983
- <sup>2</sup> SPEAR, F. F., *Radiation and Living Cells*, Chapman and Hall, London, 1953
- <sup>3</sup> DARLINGTON, C. D., *Symposium on Chromosome Breakage*, Oliver and Boyd, London, 1953, Introduction, p. viii
- <sup>4</sup> CLAUDE, A., *Biol. Symp.*, 1943, **10**, 111
- <sup>5</sup> DANIELLI, J. F., *Cytochemistry. A critical approach*, Wiley, New York, 1953, p. 139
- <sup>6</sup> DARLINGTON, C. D. and MATHER, K., *Elements of Genetics*, Allen and Unwin, London, 1949
- <sup>7</sup> HADDOCK, A., *Nature, Lond.*, 1944, **154**, 194
- <sup>8</sup> KOLLER, P. C., *Progr. Biophys.*, 1953, **4**, 195; *Symposium on Chromosome Breakage*, Oliver and Boyd, London, 1953
- <sup>9</sup> LEA, D. F., *Actions of Radiations on Living Cells*, Cambridge University Press, Cambridge, 1946
- <sup>10</sup> DARLINGTON, C. D. and LA COUR, L. F., *J. Genet.*, 1945, **46**, 180
- <sup>11</sup> SAX, K., *Cold Spr. Harb. Symp. quant. Biol.*, 1941, **9**, 93
- <sup>12</sup> LANE, G. R., *Heredity*, 1951, **5**, 1
- <sup>13</sup> CONGER, A. D. and FAIRCHILD, L. M., *Proc. nat. Acad. Sci., Wash.*, 1952, **38**, 289
- <sup>14</sup> D'AMATO, F. and GUSTAFSSON, A., *Hereditas*, 1948, **34**, 181
- <sup>15</sup> VON EULER, H. and HAHN, L., *Acta radiol., Stockh.*, 1946, **27**, 268
- <sup>16</sup> DURYEE, W. R., *J. nat. Cancer Inst.*, 1949, **10**, 735
- <sup>17</sup> ZIRKLE, R. E. and BLOOM, W., *Science*, 1953, **117**, 487
- <sup>18</sup> BACK, A. and BLOCH-FRANKENTHAL, L., *Proc. Soc. exp. Biol., N.Y.*, 1947, **66**, 366
- <sup>19</sup> GRAY, L. H., *Brit. J. Radiol.*, 1953, **26**, 609
- <sup>20</sup> REVELL, S. H., *Brit. Emp. Cancer Camp. Annu. Rep.*, 1953, **30**, 42; *Symp. Radiobiol. (Liège)*, Butterworths, London, 1955

## THE NORMAL AND PATHOLOGICAL BIOCHEMISTRY OF MITOSIS

THE problem of interpreting cytological observations in terms of biochemistry has not been solved. At present there are a number of aspects relevant to this topic which may point the way to future developments.

Biochemists and cytologists regard chromosome breakage in fundamentally different ways (*cf.* LOVELESS<sup>1</sup>). The latter have studied with the greatest precision the statistical behaviour of microscopically visible cell structures and have treated them in isolation as purely mechanical entities. The biochemist on the other hand has learned to consider the dynamic aspect of living processes and realizes that the stability of the cell structures is only apparent and that it is the result of an equilibrium of opposing processes. Water and small molecules continually enter and leave morphological structures which are always undergoing replacement of one molecule by an identical one. In adult organisms synthesis exactly counterbalances destruction so that a stationary state is maintained. In isolated cells and tissues and in growing materials there is a net gain in synthesis, but in both adult and growing organisms nearly all structures are constantly being renewed.

It is tempting to regard chromosome breakage in terms of synthesis and destruction—of anabolism and catabolism—and to ascribe the break as the visible result of interference with chromosome formation. Clearly the vital metabolites required for the formation of the DNA and protein cannot be destroyed by the small amount of energy required to produce chromosome abnormalities. These substances are present in abundance as is seen from the fact that chromosome aberrations characteristic of ionizing radiations cannot be induced by starving the cells.

Little is known concerning the origin of the energy for protein and DNA synthesis. COHEN and McGILVERY<sup>2</sup> showed that the synthesis of a molecule of hippuric acid (*i.e.* the formation of a peptide bond between glycine and benzoic acid), is provided by the degradation of one molecule of ATP to ADP\*. Very probably the same

---

\* The presence of co-enzyme A is also necessary because S-benzoyl CoA is the activated form of the benzoyl group<sup>69</sup>.

## THE NORMAL AND PATHOLOGICAL BIOCHEMISTRY OF MITOSIS

energy is used for the synthesis of polypeptides. The resynthesis of ATP occurs during certain phases of carbohydrate catabolism (see footnote p. 243) which is therefore eventually the energy source for peptide (protein) synthesis. No comparable information is available for the synthesis of chromosome material although HEVESY's<sup>3</sup> suggestion that the energy is derived from carbohydrate metabolism seems a plausible working hypothesis. Some of the chemical substances which produce chromosome breaks (*e.g.* the mustards, the peroxides and maleic hydrazide) are known to interfere with the utilization of carbohydrates and may therefore block the energy supply for the synthesis of chromosome material. It has already been observed (see p. 172) that a biochemical mechanism cannot be wholly responsible for the effect of ionizing radiations on chromosomes and that an effect on complex structures as they exist immediately before division is indicated. Nevertheless some of the nuclear effects of radiation must also occur on the biochemical level and for this reason these processes will be considered in this chapter.

### CHROMOSOMES AND DNA

It seems certain that chromosomes are composed of DNA and of protein although the exact relationship between them is unknown. In Chapter 4 some of the facts which have been established about nucleoprotein complexes isolated from cells were summarized. How nearly these materials resemble the structures present in the living cell is a matter of conjecture but there are many indications that the combination of protein and DNA is very subtle and may be extensively altered during extraction. There is no experimental evidence from which it can be decided if the chromosome strands are made up of long threads of DNA with protein adhering to them or of protein fibres with DNA attached. Differential staining tests, particularly from the giant chromosomes of the salivary gland of *Drosophila*, show that the relative amount of DNA and protein is not constant along the fibre. The disc-like structure of this chromosome (see *Figure 3*, Chapter 5) is due to variation in composition. It is not known whether the chromosomes are made up of one continuous thread consisting of an interlocking network of linear macromolecules or of a string of molecules or micelles held together by hydrogen bonds and secondary valency forces as is the case for fibrin<sup>4</sup> and of F-actin<sup>5</sup>. Many of the physical properties of chromosomes could be explained on the latter model especially if disaggregation occurred at the end of telophase. However, the perfect reproducibility of the complex

## CHROMOSOMES AND DNA

chromosome pattern—essential for genetic continuity—is difficult to reconcile with such a scheme.

A challenging problem is which components of the chromosomes carry the gene codes; is it DNA, protein or nucleoprotein? A direct answer is not possible but the following evidence indicates that the genetic material is DNA. (i) Heritable changes can be produced in bacteria by introducing so-called transforming factors isolated from organisms of the same species which carry the required genetic characteristic. Such transformations were first demonstrated<sup>6</sup> by the conversion of a non-specific, avirulent 'rough' *Pneumococcus* into virulent 'smooth' *Pneumococcus* possessing the characteristic capsule with its polysaccharide antigens. Once transformation has been effected by the addition of a chemical agent from a cell-free extract the newly acquired characteristic continues to be transmitted and a transforming substance of identical activity can be extracted from the cells far in excess of that originally added. This phenomenon has now been extended to other bacteria and to the transmission of many other heritable characteristics<sup>7, 8, 24</sup>. In every case the agent responsible was shown to be a DNA with a very high degree of specificity. (ii) Largely as the result of the work of FELIX and his colleagues<sup>9</sup> it is known that the spermheads from fish after removal of the cytoplasm consist entirely of a complex between the low molecular weight basic protein, known as protamines, and of DNA. The protamines are made up in general of only six different amino-acids. Although the number of variants theoretically possible in these simple molecules is still large it seems unlikely that they can act as gene codes and consequently DNA again appears as the genetically specific material. The fact that the only protein present in spermheads are protamines also strongly indicates that the fundamental fibre-forming material in chromosomes is DNA since the chain length of the protamines is too short for the formation of threads.

Although none of this evidence is decisive it strongly argues against the view that the genetic specificity resides in the protein moiety and that the nucleic acid serves only to maintain the protein in an expanded configuration necessary for its function as a template<sup>10</sup>. It has been claimed that, since the DNA molecule is made up of only four and in some cases five, different heterocyclic bases attached to the phosphate-sugar back-bone (see p. 142) it cannot act as an organizer for protein synthesis which requires the marshalling of 20 different amino-acids in the right sequence. By making the reasonable postulate that more than one basic residue is involved in positioning an amino-acid there are sufficient possible arrangements

## THE NORMAL AND PATHOLOGICAL BIOCHEMISTRY OF MITOSIS

to produce the specificity required for the synthesis of a polypeptide chain. Different ways in which DNA can act as a template have been worked out by CALDWELL and HINSELWOOD<sup>11</sup> and by DOUNCE<sup>12</sup>. Recently GAMOW<sup>13</sup> has proposed a particularly convincing mechanism by which the amino-acids are arranged in the gaps between four neighbouring basis groups in the twin spiral arrangement which has recently been postulated for DNA (see p. 144).

It must be stressed that the foregoing applies to the substance carrying the gene-code and not necessarily to the genes themselves. The latter are the hypothetical carriers of hereditary characteristics and are not single substances, but can most probably be considered as properties or functions of the chromosomes.

The chromosomes, like the viruses, have the power of reproduction by doubling themselves. When the threads 'split' along their length the two chromatids are believed to contain equal and identical molecules. It cannot be concluded from this that each of the daughter cells invariably contains the same amount of nucleic acid as the nucleus of the parent cell. Thus, in the first divisions of the fertilized egg, which are very rapid and do not have an intervening period for synthesis, the daughter cells share between them the total stock of DNA derived from the egg and spermatozoa although some DNA may have been synthesized even in between these rapid divisions.

During the actual growth of the embryo or young organisms, and for certain permanent repair processes in adult tissue (intestinal mucosa, haemopoietic organs, skin *etc.*) as well as for the formation of gametes, the external medium must furnish the primary units for the synthesis of nucleic acids and proteins (*i.e.* phosphate, purine and pyrimidine bases, sugars and amino-acids). If the dividing cell is deprived of these metabolites (*a*) the chromosomes do not divide perfectly; (*b*) the daughter chromosomes stick together until anaphase; (*c*) certain segments of the chromosomes are no longer stained—this indicates a decrease in the amount of DNA\* at this point, and (*d*) the spiralization of the chromosome is decreased.

Similarly, when certain plants are exposed to cold, specific regions of the chromosome no longer give the staining characteristics of DNA with Feulgen's reagent. These regions are called *heterochromatic* in contrast with the other regions referred to as *euchromatic* which continue to give the normal colour after the cold treatment. The chromosome appears therefore to be made up of regions varying

\* In model experiments ALEXANDER<sup>14</sup> showed that a synthetic nucleoprotein complex only stained with basic dyes if it contained a greater number of phosphate groups (derived from DNA) than of basic groups from the protamine. A change in the composition of the complex from 66 per cent DNA to 60 per cent DNA completely alters the staining characteristics.

## CHROMOSOMES AND DNA

in sensitivity. Heterochromatin—the material making up the heterochromatic regions—is believed by some workers<sup>15</sup> to contain an exceptionally high content of DNA in normal cells, *i.e.* cells which have not been starved by cold. MATHER<sup>16</sup> has suggested that heterochromatin provides the genes for inherited quantitative characteristics and CASPERSSON<sup>17</sup> believes it to regulate protein synthesis in the nucleus (see also BRACHET<sup>18</sup>). An oversupply of heterochromatin can accelerate the mitotic cycle and KOLLER<sup>19</sup> was able to correlate the increased division of tumour cells with alterations in the heterochromatic regions. None of these observations concerning the heterochromatic regions have been unambiguously established and the literature of the subject is highly confused. A good case can be made out, however, for the contention that the genetic control of cell division resides in the heterochromatic regions which are closely related with DNA synthesis. Chromosome breakage induced by the radiomimetic chemicals (see Chapter 7) is largely confined to the heterochromatic regions while no such specificity is apparent in the breaks produced by x-rays. This is one of the most significant differences between chemically and radiation-produced chromosome aberration.

## DNA AND RNA SYNTHESIS

At first it would appear to be a relatively simple chemical process to convert DNA to RNA and *vice versa* by oxidation or reduction reactions. On closer inspection one sees that the purine and pyrimidine make-up of these two nucleic acids is not the same; thus only RNA contains uracil, and thymine is only found in DNA. Current research indicates that the two nucleic acids are synthesized independently, although metabolic breakdown fragments of one may be incorporated in the other.

Cytochemists have tried to determine the quantity of nucleic acid present in the nucleus at different stages of mitosis. However, the results obtained with the u.v. microscope<sup>20</sup> using the wavelength at which nucleic acids absorb, do not agree with those deduced by the rather indirect but widely used Feulgen staining technique<sup>21</sup>, or by other methods<sup>22, 23, 25, 26</sup>. It cannot be decided at present if the disagreements are due to differences in the technique or due to the use of different materials. *A priori* it is not necessary to postulate that DNA is synthesized at the same stage of mitosis in different cells. Cells which do not divide (*e.g.* nerve and liver cells of vertebrates) maintain a constant amount of DNA not because the molecules remain unchanged but because the cell synthesizes as much as it destroys. In this connection Trowell's recent observation<sup>27</sup>, that

## THE NORMAL AND PATHOLOGICAL BIOCHEMISTRY OF MITOSIS

the isolated lymph glands of rats are highly radiosensitive although no cell division takes place, is very relevant.

The turnover (*i.e.* the average lifetime) of nucleic acid molecules is difficult to determine; radioactive phosphorus  $^{32}\text{P}$  is not a very suitable tracer since it is distributed between many different molecules, some of which are rapidly broken down and the phosphorus containing fragments re-used. Useful data can most readily be obtained in materials which show a relatively rapid rate of fixation of  $^{32}\text{P}$ . For example, the nucleolus of the marine alga (*Acetabularia mediterranea*) incorporates the  $^{32}\text{P}$  as RNA<sup>28</sup> while the cells of the salivary glands of *Drosophila repleta* convert  $^{32}\text{P}$  into chromosomal DNA<sup>29</sup>. In both cases the  $^{32}\text{P}$  is dispersed subsequently into the cytoplasm. TAYLOR<sup>29</sup> and others<sup>30, 31, 32, 33</sup>, using *Drosophila*, have suggested that even the cytoplasmic RNA is synthesized initially in the nucleus in the neighbourhood of the nucleolus and subsequently appears in the cytoplasm. This view probably requires modifications since recent work indicates that RNA is synthesized independently in the nucleus and the cytoplasm although at different rates, cytoplasmic synthesis being the slower.

BRACHET and SZAFARZ<sup>34</sup> have skilfully utilized the fact that the giant unicellular alga *A. mediterranea* can be divided by dissection into two fragments, one with and the other without a nucleus; the latter can survive for as long as two months. To follow the synthesis of RNA quantitatively these authors did not use  $^{32}\text{P}$  but orotic acid (labelled with  $^{14}\text{C}$ ) as the isotopic tracer. Orotic acid is a precursor for the synthesis of uracil which forms part of RNA but not of DNA and is therefore a specific indicator for RNA synthesis. Orotic acid was incorporated into the RNA of both the nucleated and non-nucleated fragment although the radioactivity in the former was approximately 1.4 times greater than that of the latter. This result was obtained whether the dissection of the alga was carried 1 or 71 days before the addition of the orotic acid. BRACHET<sup>18</sup> concludes that the cytoplasm synthesizes RNA continuously without the collaboration of the nucleus and that the nucleated fragment synthesizes more RNA in spite of the fact that it does not produce more protein than the cytoplasm<sup>35</sup>.

The discovery of independent nuclear and cytoplasmic synthesis of RNA rationalizes the observation of PAYNE *et al.*<sup>36</sup> who found that irradiation with x-rays increased the rate of incorporation of  $^{32}\text{P}$  in the RNA obtained from the cytoplasm of the liver of mice or rats, but decreased  $^{32}\text{P}$  uptake in nuclear RNA and DNA. These results cannot be reconciled with the view that the nucleolus is the sole centre of RNA synthesis.

#### DNA AND RNA SYNTHESIS

Less is known about the synthesis of DNA because it appears to be confined entirely to the nucleus and a comparison between nuclear and cytoplasmic activity is therefore not possible. All workers agree that relatively small doses of radiation inhibit synthesis of DNA while the synthesis of both protein and RNA continues normally. Thus FORSSBERG and KLEIN<sup>37</sup> found that 1250 r of x-rays inhibited mitosis of the Ehrlich ascites tumour cells from 2 until 20 hours after the irradiation. During this period the cell (*i.e.* the cytoplasm, nucleus and nucleolus) doubles in volume but the net content of DNA is not increased. The rate of synthesis of RNA is also decreased although its concentration in the cell increases with cell size.

HOWARD and PELC<sup>38</sup> using autoradiographic methods found that <sup>32</sup>P from inorganic phosphates is not incorporated into the DNA of chromosomes of the meristem of *Vicia* roots either during mitosis or for a period of several hours preceding mitosis. Synthesis of DNA takes place half-way through interphase. This phase is very radiosensitive and the cells irradiated during the middle of interphase do not synthesize DNA or undergo mitosis for 12 hours although they return to normal later; effects of doses as low as 35 r can be detected. Cells irradiated at the end of interphase divide, though after some delay. In both these cases interference with DNA synthesis seems to be responsible for the interference with mitosis. It must be emphasized that at this period of intensive DNA synthesis irradiation does not bring about chromosome aberrations with a high frequency. The most sensitive stage for chromosome breakage in *Vicia* is the end of the resting stage and the very beginning of prophase; at this time no DNA synthesis appears to take place. This difference in the time of cell sensitivity is yet another example of the absence of a correlation between mitosis arrest and chromosome fragmentation.

In general radiosensitivity of the cell does not run parallel with DNA content or with the rate of DNA synthesis. Thus in the meiosis of *Trillium erectum* pollen<sup>26, 39</sup> the radiosensitivity is increased 3·5 times between its lowest value at the pachytene stage and its highest at the diploid stage; in between these two stages the quantity of DNA in the cell is increased by 30 per cent; after the first division the amount of DNA remains constant and when the radiosensitivity reaches its lowest level the DNA content of the cell is increasing slightly.

Finally, the point made by SPARROW<sup>26</sup> should be emphasized, *viz* that one should not overlook the possibility that interference with protein synthesis, especially with that of the spindle, may result

## THE NORMAL AND PATHOLOGICAL BIOCHEMISTRY OF MITOSIS

in the production of mitotic abnormalities. Large doses of radiation undoubtedly alter the properties of proteins (see p. 137) though there is as yet no evidence that the smaller doses necessary for chromosome breaks can influence the proteins of the nucleus. The so-called primary or physiological effects (see p. 162) are possibly due to disturbances of the cytoplasmic enzymes.

### INTERRUPTION OF ENERGY SUPPLY FOR SYNTHESIS

The suppression of one or more sources of chemical energy does not appear to have been considered in connection with the effects of

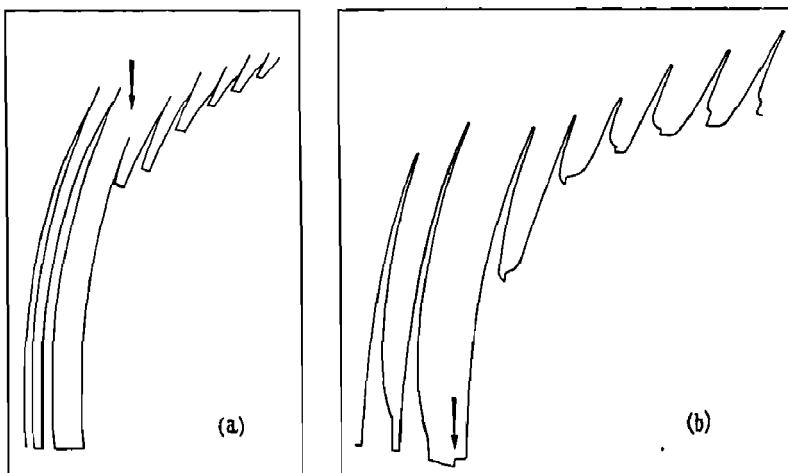


Figure 1. Similarity between action of hydrogen peroxide and  $\beta$ -rays from  $^{32}\text{P}$  on striated muscle of frog (a)  $\text{NaH}_2^{32}\text{PO}_4$ , 0.4 mc in the soln during 3 h after 2 control contractions<sup>42</sup>. The radioactive soln is washed out and the stimulations with KCl repeated. Lundsgaard contracture with inexcitability; (b)  $\text{H}_2\text{O}_2$  1.5 vol per cent for 5 min

Frog rectus abdominalis isolated in a bath of oxygenated Ringer's solution. Isotonic contractions. A quantity of concentrated KCl solution (5 per cent) is added to give good contraction, and wash after 15 to 30 sec without waiting for maximum effect. Repeat after 5 min to ensure complete muscle relaxation between excitations. Then at the time marked (see arrow) the muscle is contacted with the toxic solution; after an interval wash out solution and repeat stimulations in normal Ringer solution with KCl at 5 min intervals.

radiations, though it has become one of the most fruitful ideas in biochemical pharmacodynamics.

If a co-enzyme (*e.g.* cocarboxylase in vitamin B<sub>1</sub> deficiency) is lacking, or an enzyme system is blocked (*e.g.* by fluoride), the normal metabolism of a series of molecules is arrested, with three results: (i) certain metabolites accumulate; (ii) the energy which would have been set free by the degradation of these metabolites is no longer available; (iii) the body is forced to use other sources of

#### INTERRUPTION OF ENERGY SUPPLY FOR SYNTHESIS

energy, which tend to be quickly exhausted. This type of action is well illustrated by means of the following examples. In beri-beri, (vitamin B<sub>1</sub> deficiency) oxidation of pyruvic acid no longer takes place, and this acid accumulates in the nerve cells due to the absence of the cocarboxylase which is thiamine (vit. B<sub>1</sub>) pyrophosphate. In fluoroacetic acid poisoning there are two stages: first, fluorocitric acid is formed which then blocks the Kreb cycle and allows citric acid to accumulate<sup>40</sup>. In muscular poisoning by arsenicals, lewisite, and —SH blocking agents in general, carbohydrate metabolism, one of the main sources of energy, is blocked at various stages especially at the pyruvic acid stage, but also at the starting point<sup>41</sup>. The poisoned muscle soon exhausts its reserves of phosphocreatine, which is not being replenished; contracture develops, and the muscle no longer responds to stimulation.

Frog muscle irradiated *in vitro* with x-rays (6000–8000 r) or by <sup>32</sup>P in labelled phosphate behaves exactly like a muscle poisoned by hydrogen peroxide (*Figure 1*), by an —SH blocking agent, or by any substance which can interfere with the utilization of carbohydrates (BACQ, LECOMTE and HERVE<sup>42</sup>). The muscle is not contracted by irradiation, but if it is stimulated it does not relax completely, the contracture increases with each stimulation, and the muscle responds less and less. From this experiment it is not possible to deduce the inhibition by radiation of a particular enzyme\*, but it proves the existence of a biochemical lesion persisting after irradiation, a lesion also produced by oxidizing agents. This experiment led BACQ and HERVE<sup>44</sup> to see if the action of x-rays would be modified by oxidation inhibitors such as NaCN or NaN<sub>3</sub> (see Chapter 14).

The concept of a biochemical lesion can explain a number of experiments which show a delayed effect similar to that found with striated muscle. If frogs irradiated with 3000 to 6000 r at 23° C are cooled to 5° C<sup>45</sup>, <sup>46</sup>, 80 to 90 per cent of the animals survive for more than 3 to 4 months after irradiation, whereas controls kept at 23° C die in 3 to 6 weeks. The lesion in the cooled animals is *latent* and if they are warmed after 60 to 130 days they die. No lesion is found in the ovarian ova of amphibians 12 days after total irradiation with 3000 to 5000 r if the animals are kept at 5° C, but if they are kept at 22° C after exposure to 3000 r, all the ova are affected. If the chilled and irradiated animals are warmed after 12 days the lesions appear very quickly<sup>47</sup>.

LAMARQUE and GROS<sup>48</sup> irradiated the eggs of the silk worm

\* BARRON<sup>43</sup> has proposed a somewhat different interpretation of this experiment and many more might be suggested; for instance the inhibition of transphosphorylase of adenosine triphosphatecreatinine which according to LORAND<sup>70</sup> insures relaxation of striped muscle.

#### THE NORMAL AND PATHOLOGICAL BIOCHEMISTRY OF MITOSIS

(*Bombyx mori*) and kept them cold; when they were warmed six months later, it was found that very few of the radiation lesions had been repaired. Similarly, hibernating mammals such as squirrels or marmots are much less radiosensitive when irradiated and maintained in hibernation. On warming 2 to 4 weeks after irradiation the animals show the radiation response of animals exposed to x-rays in the non-hibernating state and die in about 10 days following a lethal dose<sup>49, 50</sup>.

Contemporary workers tend to forget that this problem was admirably stated as long ago as 1925 by P. ANCEL and P. VINTEMBERGER<sup>51</sup> who placed an unincubated hen's egg in the refrigerator for 24 hours, irradiated it with x-rays and then replaced it in the refrigerator. Three days later no lesion was to be seen. If, however, an irradiated egg was incubated during the 3 days following irradiation many lesions were found. *The factor which reveals the lesion caused by irradiation is cellular activity.* Ancel and Vintemberger doubted the value of direct histological examination for determining differences in the radiosensitivity of cells. The authors reached the following prophetic conclusions which are still valid: 'Three essential points must be clearly distinguished: (i) the radiation lesion; (ii) the factors bringing about the manifestation of the lesion; (iii) healing factors. It must not be forgotten that the lesions revealed by the microscope are the results of the combined, and sometimes antagonistic action of these factors.' It is only necessary to describe the lesion as biochemical to bring these conclusions formulated 30 years ago into line with present concepts.

The limitation of the energy reserves of irradiated cells has also already been mentioned by VINTEMBERGER in 1930<sup>52</sup>. He wrote: 'The duration of survival of an irradiated cell is inversely proportional to its activity after irradiation.' As long as an irradiated muscle is not stimulated, or irradiated amphibians or eggs are kept at a low temperature, nothing is to be seen. The muscle must be stimulated and the frogs or eggs warmed, in other words, metabolism and oxygen consumption must be increased, to make the characteristic lesions appear. GRAY's conclusion<sup>53</sup>, that 'metabolism plays an essential role in the development of injury to the structural components of the nuclei studied' is exactly in line with the biochemical lesion as defined by R. A. PETERS and developed by us within the framework of radiobiology. The results obtained by DURYEE<sup>47</sup> not only conform with the general idea which emerges from recent work with protectors against radiation, but also with the inability of cytophysiologists to explain the nuclear lesions solely by the action of radiations on the nucleus and chromosomes (see p. 70).

#### INTERRUPTION OF ENERGY SUPPLY FOR SYNTHESIS

The higher the rate of metabolism, the more rapidly are abnormal metabolites formed\*, and the more rapidly are the small stores of chemical energy dissipated.

#### ALTERATION OF PERMEABILITY

The suggestion that radiation injuries bring about an alteration in the permeability of certain intracellular structures seems very promising. BACQ and HERVE<sup>55</sup> have already developed it to some extent, and SPARROW<sup>26</sup> mentions it briefly. On the cellular scale, biochemical processes are controlled by the very exact localization of the enzymes and substrates, which prevents them from coming into contact, although they exist within the same cell.

A study *in vitro* of the activity of crystallized enzymes is an experiment in pure chemistry which reveals little of what really happens in the cell. The cellular environment is extremely heterogeneous. A very great number of structures co-exist within the cellular membrane, and each possesses its own system of enzymes which are not free and soluble in the cytoplasm. In addition to the nucleus with its membrane, the chromosomes and the nucleolus, there are other morphologically distinct structures in the cell, notably the mitochondria, which can be seen to be mobile in tissue cultures. Their shape and position vary continually; they can be seen approaching the nuclear membrane, and it seems probable that direct exchanges take place between the mitochondria and the nucleus<sup>56</sup>. The submicroscopic structures, the microsomes of various sizes are rich in enzymes and in phospholipids, which probably constitute their 'membrane'. After selective crushing of the cells by HOGEBOOM's modification<sup>59</sup> of CLAUDE'S<sup>57, 58</sup> method (*i.e.* rupture of the cell membrane in a hypertonic sucrose medium), the first centrifugation at low speed separates the heaviest elements (nuclei and fragments of membrane), the second isolates the mitochondria, after which lighter fractions, the microsomes, are removed by successive ultracentrifugation. The supernatant, making up the apparently homogenous unorganized fraction of the cytoplasm, contains only a small proportion of the enzymatic equipment of the cells. This method of selective ultracentrifugation has not so far been used widely for the separation of the intracellular substrates. These must of course be separated from their enzymes and may be localized. For example, at least 60 per cent of the acid phosphatase is associated with the mitochondria; glycerophosphate, which is a substrate of acid phos-

---

\* The Lundsgaard contracture (see *Figure 1*) seems also to be due to the accumulation of a poison causing contracture<sup>54</sup>.

## THE NORMAL AND PATHOLOGICAL BIOCHEMISTRY OF MITOSIS

phatase, does not pass through the 'membrane' of isolated mitochondria<sup>60</sup>. The cholinesterases of the liver of rats and guinea-pigs are largely localized in structures smaller than the mitochondria<sup>61</sup>. The alkaline phosphatase is concentrated in the mitochondria. As DE DUVE and his collaborators<sup>60</sup> say, the cell is divided into compartments.

These observations explain several well-known facts in animal and plant biochemistry which could only be vaguely interpreted before. Notably the co-existence in the same tissue of substrates and their enzymes, e.g. acetylcholine and cholinesterase can now be understood. The most striking example of this separation is probably that of phenoloxidase in certain fungi and the potato tuber. In a normal cell it is separated from the phenolic substances which it oxidizes into black melanin via the intermediary of red quinones. Young wild mushrooms of *Russula nigricans* are white, but if they are cut, that is, if the cellular structures are injured, the enzyme and substrate can come into contact and the mushrooms become black. For this reason the track of a parasite within the mushroom shows up black, and ageing or cellular necrosis, is always accompanied by blackening. These fungi provide a simple test, visible within a few hours for determining if the barrier between the enzyme and the substrate has been broken. BACQ and HERV<sup>55</sup> have performed preliminary experiments with this material. Irradiation with 20,000 r at 60 kV, or 150,000 r of soft x-rays does not change the colour or the enzymatic behaviour of the tissues of *Boletus calopus* and *B. erythropus*, but the effect of x-rays can be seen in *Russula nigricans*. For instance, 7000 r (contact therapy apparatus, 50 kV × 2 mA, focal distance 1 cm; 7000 r/min) causes blackening to a depth of 3 to 4 mm, clearly visible 20 hours after irradiation, whereas the blackening of control zones is only 1 to 1.5 mm in depth. This material deserves detailed investigation as it lends itself to quantitative studies. Similar experiments with potato tubers have been published by SUSSMAN<sup>62</sup>.

The increase in the two desoxyribonucleases in the urine of rats after irradiation<sup>63</sup> might be put forward as another proof of the mobilization of intracellular enzymes. Others might be found in the urine if a systematic search were made\*. The alkaline phosphatase in the plasma of rats increases by about 25 per cent during the first day following irradiation with 500 to 600 r, decreases to 35 per cent below that of controls on the third day, and then remains low<sup>65</sup>. This enzyme comes from the tissues, and x-rays

---

\* Enzymes are also found in the urine following certain kinds of poisoning; e.g. catalase in uranium poisoning<sup>64</sup>.

#### ALTERATION OF PERMEABILITY

either cause increased synthesis of this phosphatase or increased liberation of the enzyme molecules already present. It is impossible to choose between these interpretations, because we cannot determine the phosphatase balance.

The displacement by irradiation of enzymes normally associated with certain intracellular structures might be the first stage in their inactivation, since 'liberated' enzymes might come into contact with cellular proteases which do not normally touch them.

The idea of breakdown of the intracellular structures is only an extension to the microsomes of events which can be seen in the chromosomes. It accounts for the *increase* in oxygen consumption by the bone-marrow very soon after irradiation, and for the liberation in the plasma of a factor which accelerates haemoglobin synthesis by the reticulocytes in dogs\*. This aspect is fully discussed in Chapter 10.

*Summary*—It has already been stressed (see pp. 74 and 173) that the greater effectiveness of more densely ionizing radiations, which is shown by so many living systems and which distinguishes biological systems from simple *in vitro* reactions, can best be interpreted as a breakdown of an 'organized structure'. According to the classical cytologists the 'organized structure' is the chromosome which has to be broken. This concept is too simple to explain even the appearance after irradiation of chromosome aberration and it can certainly not explain many other radiobiological effects. If it is postulated that the 'organized structures' which are damaged by radiation include intracellular barriers the following findings general to radiobiology can be understood:

(i) A small dose of radiation affects many more molecules, than could be explained on the number of free radicals formed, by allowing enzymes and substrates normally kept separate to interact, *i.e.* the ionizing radiations open the 'flood gates' of a damned-up system.

(ii) Enzymatic activity in the blood and tissue fluids is in many cases increased and not decreased immediately after irradiation.

---

\* LUDEWIG and CHANUTIN<sup>66</sup> conclude that a dose of 500 r to the whole body in rats does not affect the intracellular distribution of enzymes in the liver. Also MAXWELL and ASHWELL<sup>67</sup> found that in a homogenate of mouse spleen the adenosinetriphosphatase activity of all the individual fractions (nuclei, mitochondria, microsomes and supernatant substance) is about 3 times greater after irradiation with 640 r than in controls, which would make it appear that there is no 'liberation' of this enzyme system, which is especially abundant in the microsomes. However, the number of experiments on which this study is based is small, and little attention was paid to the time-interval between irradiation and removal of the spleen.

## THE NORMAL AND PATHOLOGICAL BIOCHEMISTRY OF MITOSIS

(iii) The relative biological effect (RBE) generally increases with an increase in the specific ionization of the radiation used because the intracellular barriers cannot be destroyed by a single ionization and it is necessary for much energy to be deposited in a small area, i.e. a number of ionizations widely dispersed have no effect and are wasted.

### REFERENCES

- <sup>1</sup> LOVELESS, A., *Symposium on Chromosome Breakage*, Oliver and Boyd, London, 1953
- <sup>2</sup> COHEN, P. P. and MCGILVERY, R. W., *J. biol. Chem.*, 1947, **169**, 119
- <sup>3</sup> HEVESY, G., *Symposium on Radiobiology*, Wiley, New York, 1952, p. 189
- <sup>4</sup> PORTER, K. R. and HAWN, Z., *J. exp. Med.*, 1949, **90**, 225
- <sup>5</sup> PERRY, S. V. and REED, R., *Biochim. biophys. Acta*, 1947, **1**, 379
- <sup>6</sup> GRIFFITH, F., *J. Hyg., Camb.*, 1928, **27**, 113
- <sup>7</sup> AVERY, O. T., MACLEOD, C. M. and McCARTY, M., *J. exp. Med.*, 1944, **79**, 137
- <sup>8</sup> EPHRUSSI-TAYLOR, H., *Cold Spr. Harb. Symp. quant. Biol.*, 1951, **16**, 445
- <sup>9</sup> FELIX, K., *Experientia*, 1952, **8**, 312
- <sup>10</sup> HAUROWITZ, F., *The Chemistry and Biology of Proteins*, Academic Press, New York, 1950
- <sup>11</sup> CALDWELL, P. G. and HINSHELWOOD, C., *J. chem. Soc.*, 1950, 3156
- <sup>12</sup> DOUNCE, A. L., *Enzymologia*, 1952, **15**, 251
- <sup>13</sup> GAMOW, G., *Nature, Lond.*, 1954, **173**, 318
- <sup>14</sup> ALEXANDER, P., *Biochim. biophys. Acta*, 1953, **10**, 595
- <sup>15</sup> LA COUR, L. F., *Heredity*, 1951, **5**, 37
- <sup>16</sup> MATHER, K., *Proc. roy. Soc.*, 1944, **B132**, 308
- <sup>17</sup> CASPERSSON, T., *Symp. Soc. exp. Biol.*, 1947, **1**, 127
- <sup>18</sup> BRACHET, J., 'Le rôle des Acides Nucléiques dans la Vie de la Cellule et de l'Embryon', *Actualités Biochimiques*, No. 16, Desoer, Liège et Masson, Paris, 1952
- <sup>19</sup> KOLLER, P. C., *Nature, Lond.*, 1943, **157**, 244
- <sup>20</sup> CASPERSSON, T., *Cell Growth and Cell Function*, Norton, New York, 1950
- <sup>21</sup> PASTEELS, J. and LISON, L., *C.R. Acad. Sci., Paris*, 1950, **230**, 780
- <sup>22</sup> POLLISTER, A. W., SWIFT, H. and ALFERT, M., *J. cell. comp. Physiol.*, Suppl. No. 1, 1951, **38**, 101
- <sup>23</sup> VENDRELY, R. and VENDRELY, C., *Experientia*, 1949, **5**, 327
- <sup>24</sup> BOIVIN, A., DELAUNAY, A., VENDRELY, R. and LEHOULT, Y., *C.R. Acad. Sci., Paris*, 1945, **221**, 718
- <sup>25</sup> DAVIDSON, J. N., *Cold Spr. Harb. Symp. quant. Biol.*, 1947, **12**, 50
- <sup>26</sup> SPARROW, A. H., *Ann. N.Y. Acad. Sci.*, 1951, **51**, 1508
- <sup>27</sup> TROWELL, O. A., *Brit. J. Radiol.*, 1953, **26**, 302
- <sup>28</sup> STICH, H. and HAMMERLING, J., *Z. Naturf.*, 1953, **B8**, 329
- <sup>29</sup> TAYLOR, J. H., *Science*, 1953, **118**, 555
- <sup>30</sup> MARSHAK, A., *J. cell. comp. Physiol.*, 1948, **32**, 381
- <sup>31</sup> JEENER, R. and SZAFARZ, D., *Arch Biochem.*, 1950, **26**, 54
- <sup>32</sup> BARNUM, C. P. and HUSEBY, R. A., *ibid.*, 1950, **29**, 7

REFERENCES

- <sup>33</sup> SMELLIE, R. M. S., McINDOE, W. M., LOGAN, R., DAVIDSON, J. N. and DAWSON, I. M., *Biochem. J.*, 1953, **54**, 280
- <sup>34</sup> BRACHET, J. and SZAFARZ, D., *Biochim. biophys. Acta*, 1953, **12**, 588
- <sup>35</sup> BRACHET, J. and CHANTRENNNE, H., *Nature, Lond.*, 1951, **168**, 950
- <sup>36</sup> PAYNE, A. H., KELLY, L. S. and ENTENMAN, C., *Proc. Soc. exp. Biol.*, 1952, **81**, 698
- <sup>37</sup> FORSSBERG, A. and KLEIN, G., *Exp. Cell Res.*, 1954, **6**, 211
- <sup>38</sup> PELC, S. R. and HOWARD, A., *Aarhus Symp.*, 1953; in press
- <sup>39</sup> MOSES, M. J. and STEELE, R., *Brit. J. Radiol.*, 1952, **25**, 182
- <sup>40</sup> PETERS, R. A., *Proc. roy. Soc.*, 1952, **B139**, 143
- <sup>41</sup> PETERS, R. A., *Symposium sur le cycle tricarboxylique*, Second International Congress of Biochemistry, Paris, 1952, p. 64
- <sup>42</sup> BACQ, Z. M., LECOMTE, J. and HERVE, A., *Arch. int. Physiol.*, 1949, **67**, 142
- <sup>43</sup> BARRON, E. S. G., *Symposium on Radiobiology*, Wiley, New York, 1952, p. 236
- <sup>44</sup> BACQ, Z. M. and HERVE, A., *Brit. J. Radiol.*, 1951, **24**, 617
- <sup>45</sup> PATT, H. M. and SWIFT, M. N., *Amer. J. Physiol.*, 1948, **155**, 388
- <sup>46</sup> PATT, H. M., SWIFT, M. N. and TYREE, E. B., *Fed. Proc.*, 1948, **7**, 90
- <sup>47</sup> DURYEE, W. R., *J. nat. Cancer Inst.*, 1949, **10**, 735
- <sup>48</sup> LAMARQUE, J. P. and GROS, C., *Brit. J. Radiol.*, 1945, **18**, 293, and Seventh International Congress of Radiology, Copenhagen, 1953
- <sup>49</sup> SMITH, F. and GRENNAN, M. M., *Science*, 1951, **113**, 686
- <sup>50</sup> DOULL, J., PETERSEN, D. F. and DUBOIS, K. P., *Fed. Proc.*, 1952, **11**, 340
- <sup>51</sup> ANCEL, P. and VINTERMBERGER, P., *C.R. Soc. Biol., Paris*, 1925, **92**, 517
- <sup>52</sup> VINTERMBERGER, P., *Arch. Anat., Strasbourg*, 1930-31, **12**, 299-464
- <sup>53</sup> GRAY, L. H., *Progr. Biophys.*, 1951, **2**, 240
- <sup>54</sup> BACQ, Z. M. and GODEAUX, J., unpublished results
- <sup>55</sup> BACQ, Z. M. and HERVE, A., *Bull. Acad. Méd. Belg.*, 1952, 6th series, **18**, 13
- <sup>56</sup> FREDERIC, J. and CHEVREMONT, M., *Arch. Biol., Paris*, 1952, **63**, 109
- <sup>57</sup> CLAUDE, A. and POTTER, V., *J. exp. Med.*, 1943, **77**, 345
- <sup>58</sup> CLAUDE, A., *ibid.*, 1946, **84**, 51
- <sup>59</sup> HOGEBOOM, G. H., SCHNEIDER, W. C. and PALLADE, G. E., *J. biol. Chem.*, 1948, **172**, 619
- <sup>60</sup> DE DUVE, Chr., BERTHET, J., BERTHET, L. and APPELMANS, F., *Nature, Lond.*, 1951, **167**, 389
- <sup>61</sup> GOUTIER, R. and GOUTIER-PIROTTE, M., *Arch. int. Physiol.*, 1954, **62**, 151
- <sup>62</sup> SUSSMAN, A. S., *J. cell. comp. Physiol.*, 1953, **42**, 273
- <sup>63</sup> KOWLESSAR, O. D., ALTMAN, K. I. and HEMPELMANN, L. H., *Nature, Lond.*, 1953, **172**, 867
- <sup>64</sup> VOEGTLIN, C. and HODGE, H. C., *The Pharmacology and Toxicology of Uranium Compounds*, McGraw Hill, New York, 1949
- <sup>65</sup> LUDEWIG, S. and CHANUTIN, A., *Amer. J. Physiol.*, 1950, **163**, 648
- <sup>66</sup> LUDEWIG, S. and CHANUTIN, A., *Arch. Biochem.*, 1950, **29**, 441
- <sup>67</sup> MAXWELL, E. and ASHWELL, G., *ibid.*, 1953, **43**, 389
- <sup>68</sup> CHANTRENNNE, H., *J. biol. Chem.*, 1951, **189**, 227
- <sup>69</sup> SCHECHTER, D. and TAGGART, J. V., *ibid.*, 1953, **203**, 905
- <sup>70</sup> LORAND, L., *Nature, Lond.*, 1953, **172**, 1181

## CHEMICAL SUBSTANCES WHICH SIMULATE THE BIOLOGICAL EFFECTS OF IONIZING RADIATIONS

THE biological activity of certain chemical substances, often referred to as 'radiomimetic'<sup>1</sup> because they produce many of the biological *end-effects* observed after treatment with ionizing radiations, is closely related to the study of radiobiology and justifies the inclusion of this subject in this book.

Effects such as arrest of mitosis<sup>2</sup> and induction of tumours<sup>3</sup> have been known for many years to be produced by chemical substances as well as by ionizing radiations, but it was the discovery by Miss C. AUERBACH<sup>4</sup> in 1943 that the chemical warfare agent, mustard gas,  $S(CH_2CH_2Cl)_2$ , is a powerful mutagenic agent which for the first time revealed the possibility that the biological effects of ionizing radiations could also be produced by chemical agents.\* The production of gene-mutations had until this time been considered as a characteristic property of ionizing radiations, and its interpretation by the target theory (see p. 66) appeared to provide a consistent and convincing explanation on purely physical lines which was extended to many other radiobiological phenomena. Miss Auerbach's remarkable observation, coming shortly after Dale's demonstration that enzymes could be inactivated with x-rays by indirect action (see p. 47), initiated a re-interpretation of radiobiology along more chemical lines. The rapid development of our subject in the last ten years bears testimony to the fruitfulness of this new approach.

Radiomimetic substances may be defined as chemicals which act on the cell *nucleus* during the resting stage to bring about true chromosome breakage and gene-mutations, and which induce tumours. LOVELESS<sup>6</sup> has defined radiomimetic activity as a highly specific effect upon the resting cell producing an alteration of the genetic material which is revealed by chromosome breakage and rearrangement in subsequent division. In general, these effects become

---

\* Conversely, it suggested to one of us (Z.M.B.) that the amines which inhibit mustard gas action might also be used to counteract the effects of purely physical agents such as ionizing radiations and led to the discovery of some of the protective agents which are discussed in Chapter 14.

## CHEMICALS SIMULATING EFFECTS OF IONIZING RADIATIONS

apparent at relatively low concentration when the substances do not bring about toxic effects. By increasing both the duration of treatment and the concentration of the agent it is possible to kill both dividing and non-dividing cells.\* A number of substances are known which produce chromosome breakage, and in some cases also gene-mutations, without fulfilling the requirement for radiomimetic action†. For example, some mitotic poisons (*e.g.* urethane, the first chemical which had been shown to produce permanent chromosome abnormalities<sup>8</sup>) affect the cytoplasm<sup>9</sup> where they bring about disturbances (*e.g.* enzyme inhibition), the consequences of which become apparent in the nucleus and result in the appearance of chromosome fragmentation. In view of the limited data available concerning the mode of action of mitotic poison, the classification of a particular substance as radiomimetic is bound to be somewhat arbitrary. Following the criteria laid down by KOLLER<sup>10</sup> and his colleagues<sup>11</sup> we shall restrict this term to describe chemical substances containing the groups shown in *Table I*. A case could be made out for including certain organic peroxides<sup>12</sup>, and amino-stilbene and some of its derivatives<sup>13</sup>. The former, however, have not been found carcinogenic or growth-inhibitory, while the latter have not yet been shown to be mutagenic. Some authors<sup>14</sup> have referred to the carcinogenic hydrocarbons as radiomimetic largely as a result of DEMEREC's<sup>15</sup> finding that they are mutagenic; since DEMEREC<sup>16</sup> has now revised his conclusions the hydrocarbons are not included amongst the radiomimetics.

In addition to the mutagenic, carcinogenic and chromosome-breaking activity, radiations and the radiomimetic substances also share equally the ability to produce:

- (i) local greying or bleaching of hair<sup>17, 18</sup>
- (ii) characteristic acute and chronic degenerative changes in the bone-marrow, intestinal mucosa and testis<sup>19</sup>
- (iii) an identical coagulation defect<sup>20</sup>

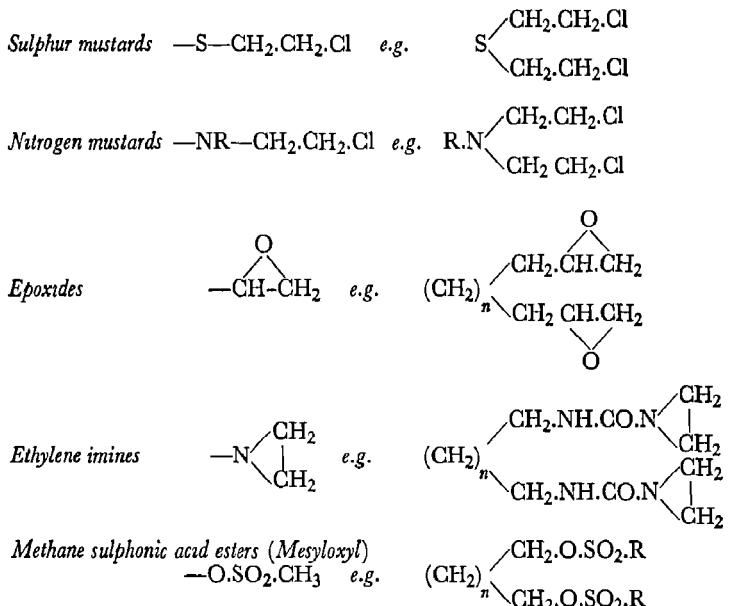
\* The toxic action of the radiomimetics is not confined to the nucleus. The relative sensitivity of nucleus and cytoplasm was determined in amoeba by HARRIS, LAMERTON, ORD and DANIELLI<sup>7</sup> by removing the nucleus and irradiating it separately, followed by reconstitution with the cytoplasm. To kill the organism a 10 times greater dose of nitrogen mustard (*vide infra*) has to be applied to the cytoplasm than to the nucleus. For x-rays the sensitivity differs by a factor of 2.5.

† There are substances, notably cortisone and the folic acid antagonists which bring about chromosome breakage without being mutagenic or carcinogenic<sup>7a</sup>. The lesions produced by vitamin antagonists suggest a working hypothesis for some of the biochemical effects produced by radiation which is similar to a mechanism proposed by BINET<sup>7b</sup> for the nitrogen mustards. The free radicals produced by the radiations transform a vitamin or growth factor into an antivitamin, the influence of which is felt at a period after the irradiation is complete, and perhaps even at a site which has not been irradiated (*cf.* poison theory discussed on p. 72).

CHEMICALS SIMULATING EFFECTS OF IONIZING RADIATIONS

- (iv) the suppression of antibody formation
- (v) pronounced growth-inhibitory\* activity as judged against the growth of various animal tumours<sup>21</sup>.

*Table I. Radiomimetic Compounds*



THE CHEMISTRY OF THE RADIOMIMETIC SUBSTANCES

The recognition that compounds containing the groups shown in *Table I* as well as fulfilling a number of other requirements (*vide infra*) are radiomimetic, originates from the discovery that mustard gas was cytotoxic and mutagenic. It was soon realized that closely related compounds, the so-called nitrogen mustards, possessed the same biological activity and the extensive literature on this subject has been fully reviewed by PHILIPS<sup>22</sup> and BACQ<sup>61</sup>. From experiments designed to determine the optimum requirements for the cytotoxic action of these substances HADDOCK, KON and ROSS<sup>23</sup> found in 1948 that only substances containing two halogenoalkyl groups per molecule were active in the growth-inhibition test used. Other workers reached this same conclusion independently<sup>24</sup>. ROSS<sup>25</sup> further found that for active compounds the halogenoalkyl groups must have minimum

\* In general, cells which are specially active in synthesizing protein and DNA and which have the ability to undergo rapid proliferation are particularly sensitive to these chemicals in the same way as they are to ionizing radiations (see p. 156).

## THE CHEMISTRY OF THE RADIOMIMETIC SUBSTANCES

chemical reactivity as measured by the rate of hydrolysis. Once these requirements had been met the structure of the remaining part of the molecule appeared to be capable of influencing the biological activity only quantitatively. Thus biological activity was found in all compounds of the general type  $\text{RN}(\text{CH}_2\text{CH}_2\text{Cl})_2$  whether they are basic, acidic or neutral, water or oil soluble; active compounds have been obtained where R consists of almost every type of aliphatic, aromatic or heterocyclic structure. The essential feature for activity was clearly the chemical reactivity of the two mustard groupings. The halogen atoms are very reactive and confer on the mustards the ability to act as alkylating agents (*vide infra*). It was an obvious next step to look for biological activity in compounds containing groups having the same chemical reactivity as the mustards. Independently different workers (see reviews by HADDOCK<sup>26</sup> and ALEXANDER<sup>27</sup>) found that compounds containing two or more epoxide, ethylene imine or mesyloxy groups<sup>64</sup> were cytotoxic and inhibited tumour growth in experimental animals\*. Further investigation showed that these substances were carcinogenic<sup>26</sup>, mutagenic<sup>28</sup> and produced chromosome fragmentation<sup>6, 11, 29</sup>. All other tests also showed that these alkylating agents possess true radiomimetic activity as defined above.

*Need for more than one functional group*—Since the major interest of the radiomimetic substances is related to their possible use as chemotherapeutic agents against cancer they are generally examined as growth-inhibiting agents in animals. The extensive work of HADDOCK<sup>21</sup> and of ROSE and his colleagues<sup>30</sup> has clearly demonstrated that in general only compounds containing two or more alkylating groups are active. Out of the several hundred compounds which were found to be active as growth inhibitors only three were monofunctional and all of these contained a second centre having unusual physicochemical characteristics.

Subsequently it was found that monofunctional derivatives as a class were capable of producing certain radiomimetic effects<sup>6, 31</sup> but only at much higher doses. Their ineffectiveness as growth inhibitors is due to the fact that the toxicity of the alkylating agents is too great to permit the administration to animals of the greatly increased doses needed with monofunctional compounds. LOVELESS<sup>6</sup> found that to produce a comparable amount of chromosome

\* A further condition for activity has become apparent from studies of the mesyloxy series<sup>64</sup>. The spatial configuration of the two reactive groups has to be such that they can react at two closely adjacent sites (see p. 205) or with the same group (e.g. with an amine  $\text{X}-\text{R}-\text{X}+\text{R}_1\text{NH}_2 \rightarrow \text{R}_1\text{N}-\text{R}-\text{X}$ ). The same requirement may also be applicable to the bis-epoxides and mustards, but for these the experimental evidence is not so decisive<sup>27</sup>.

CHEMICALS SIMULATING EFFECTS OF IONIZING RADIATIONS

damage in *Vicia* it was almost invariably necessary to use approximately 50 times the concentration of the monofunctional as compared with a similar bifunctional compound. Monofunctional derivatives have not been studied in the same detail as the corresponding bifunctional agents and it is not known whether the same ratio of effectiveness applies to different radiomimetic effects. For example, STEVENS and MYLROIE<sup>32</sup> found that monofunctional derivatives of sulphur mustard had the same mutagenic activity as the bifunctional parent substance, although Haddow failed to detect growth inhibition with the monofunctional mustard,  $C_2H_5.S.CH_2CH_2Cl$ .

With the rather limited data available the tentative conclusion seems justified, that monofunctional alkylating agents are radiomimetic but that very often the presence of a second group enhances the activity very greatly. Because of the toxicity of the compounds radiomimetic activity can in general only be detected in animals with the polyfunctional derivatives.

*Nature of the chemical reactivity*—The groups shown in *Table I*, which confer radiomimetic activity are 'electrophilic' alkylating agents since they react most readily with structures which are rich in electrons (see *Table II*). For example, these agents will readily alkylate

*Table II*

<i>Groupings</i>	<i>Reactive Form</i>	<i>Less-reactive Form</i>
<i>Organic acid</i> . . . . .	$R.COO^-$ .	$R.COOH$
<i>Hydroxy or phenolic compound</i> . . . . .	$R.O^-$	$R.OH$
<i>Sulphydryl</i> . . . . .	$R.S^-$	$R.SH$
<i>Amine</i> . . . . .	$R_3N$	$R_3HN^+$
<i>Thio ether</i> . . . . .	$R.S.R.$	$\overline{R}$
<i>Phosphorus compounds</i> . . . . .	$PR_3$ ( <i>trivalent</i> )	$OPR_3$ ( <i>pentavalent</i> )

carboxylic acids if present in the ionized form (*i.e.* when the pH of the solution is greater than the pK) while the reverse applies to amines which react in their un-ionized form (*i.e.* when the pH is less than the pKa). The nature of the reaction products are summarized in *Table III*.

Both in nucleic acids and proteins there are a number of different centres which are capable of being alkylated by the radiomimetic compounds (see *Figures 1 and 2*); in proteins at pH 7 the groups in the most reactive form are the carboxyl and terminal amino groups,

## THE CHEMISTRY OF THE RADIOMIMETIC SUBSTANCES

Table III. Reactions of Nucleophilic Alkylating Agents

Type of compound	Reaction with	
	ionized carboxyl group (R—COO <sup>-</sup> Na <sup>+</sup> )	amino group (R—NH <sub>2</sub> )
Epoxide $\text{CH}_2-\text{CH}-\text{O}-$	$\text{R}-\text{COO} \cdot \text{CH}_2-\text{CH}-$ $\quad\quad\quad  $ $\quad\quad\quad \text{OH}$ + NaOH	$\text{R}-\text{NH} \cdot \text{CH}_2-\text{CH}-$ $\quad\quad\quad  $ $\quad\quad\quad \text{OH}$
Ethylene imine $\text{CH}_2-\text{CH}_2-\text{N}-$	$\text{R}-\text{COO} \cdot \text{CH}_2-\text{CH}_2-\text{NH}-$ + NaOH	$\text{R}-\text{NH} \cdot \text{CH}_2-\text{CH}_2-\text{NH}-$
Mustard gas group $\text{Cl} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{S}-$	$\text{R}-\text{COO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{S}-$ + NaCl	$\text{R}-\text{NH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{S}-$ + HCl
Nitrogen mustard group $\text{Cl} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{N} <$	$\text{R}-\text{COO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{N} <$ + NaCl	$\text{R}-\text{NH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{N} <$ + HCl
Mesyloxy group $\text{CH}_3 \cdot \text{SO}_2 \cdot \text{O} \cdot \text{R}'-$ (e.g. R' is $(\text{CH}_2)_n$ )	$\text{R}-\text{COO} \cdot \text{R}'-$ + Na <sup>+</sup> —O·SO <sub>2</sub> ·CH <sub>3</sub>	$\text{R}-\text{NH} \cdot \text{R}'-$ + CH <sub>3</sub> ·SO <sub>2</sub> ·O <sup>-</sup> H <sup>+</sup>

while in nucleic acids the phosphate as well as all the amino are in the form most favourable for reaction\*.

The rate with which different groups react with these alkylating agents varies very widely. The relative affinity of different groups for the mustards can best be expressed as a 'competition factor' and OGSTON<sup>36</sup> has determined this for a large number of anions. Those showing the highest reactivity contain —S<sup>-</sup> groups. Thus the competition factor for the dithiophosphate ion is ten thousand times as great as that of the acetate ion. The reactivity of the corresponding RSH compounds will be much smaller, but will probably still be significant. In proteins the pK of the —SH group is

\* Unexpectedly it was found<sup>27</sup> that the radiomimetic alkylating agents react in proteins preferentially with the amino groups of lysine and esterification of the carboxyl groups occurs to a much smaller extent. This anomaly may be due to the fact that the steric restrictions imposed on these groups in proteins modifies their chemical reactivities. ALEXANDER<sup>33</sup> *et al.* suggested that the internally neutralized carboxyl and amino groups, —NH<sub>3</sub><sup>+</sup> ... OOC—, behave chemically as if they existed in the isomeric un-ionized form, —NH<sub>2</sub> ... HOOC—. The reactivity of the different groups in nucleic acids is as expected; GULLAND *et al.*<sup>34</sup> showed that the predominant reaction of mustard gas was esterification of the phosphate groups and this observation was extended by ALEXANDER<sup>35</sup> to the other radiomimetic groupings. A small amount of reaction only would be expected with the amino groups in nucleic acids since these are extremely weak bases and are, therefore, not very reactive towards electrophilic alkylating agents<sup>25, 36</sup> even when un-ionized.

CHEMICALS SIMULATING EFFECTS OF IONIZING RADIATIONS

approximately 10 and at pH 7, therefore, they are wholly in the less-reactive form. Even so protein SH groups do react to some extent

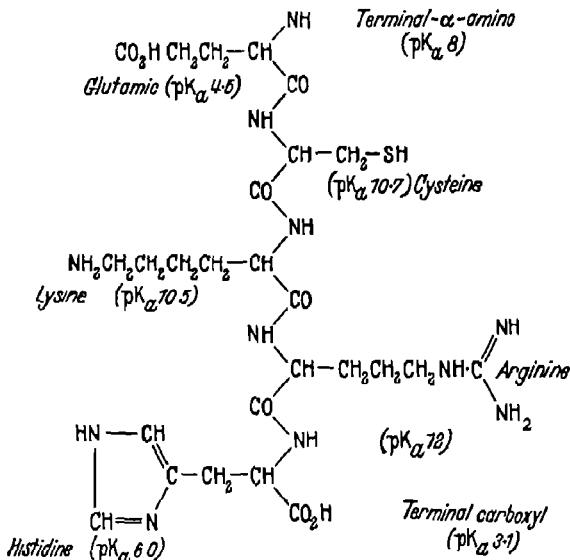


Figure 1. Diagrammatic representation of the different groups in proteins capable of reacting with the radiomimetic alkylating agents

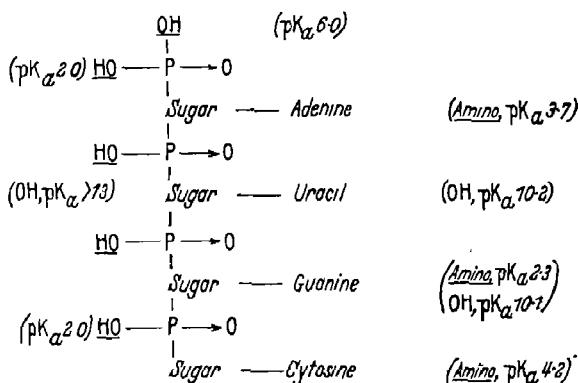


Figure 2. Diagrammatic representation of the different groups in nucleic acids capable of reacting with the radiomimetic alkylating agents

at neutrality<sup>5</sup> and in alkaline solutions this will be the predominant reaction (Figure 3).

ALEXANDER<sup>27</sup> found that DNA and polyelectrolytes had competition factors which were much higher than would be expected from the reactivity of the individual groups present. It would appear as if the presence of several reactive centres close together

## THE CHEMISTRY OF THE RADIOMIMETIC SUBSTANCES

enhances the reactivity of the individual group. These results support the view (see p. 205) that the biological mechanism of the radiomimetic alkylating agents depends upon reaction with ionized macromolecules such as DNA.

Alkylating agents which are not electrophilic (*i.e.* which do not react specifically with electron-rich groups) have not been found to possess radiomimetic properties. For example, diazo-methane

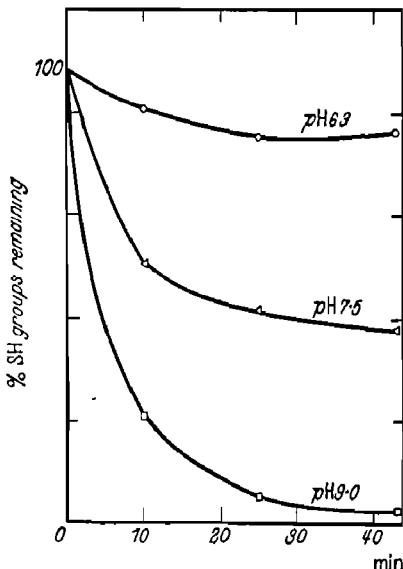


Figure 3. Rate of reactions of mustard gas with the SH groups of catile, lens denatured proteins in solutions adjusted to different pH values<sup>5</sup>

( $\text{CH}_2\text{N}_2$ ) and its derivatives which readily esterify undissociated carboxyl and hydroxyl groups are inactive. Compounds which readily alkylate  $-\text{NH}_2$  and  $-\text{SH}$  groups and do not react with anions (*e.g.*  $-\text{COO}^-$ ) such as isocyanates, halogenopyrimidines and halogeno-2, 4-dinitrobenzene, show no biological activity. This indicates that the biologically significant reaction of the radiomimetic alkylating agents is reaction with ionized acid groups.

## BIOLOGICAL ACTION OF THE RADIOMIMETIC SUBSTANCES

The impressive parallelism between the biological end-effects produced by the radiomimetic agents and ionizing radiations does not imply that the mechanism by which these changes are brought about is necessarily the same. From a study of chromosome fragmentation which will be described below, KOLLER and CASARINI<sup>37</sup> were led to conclude that 'it would be a gross error to infer a similarity of the mode of action of nitrogen mustard and x-rays based on the similarity of some end-products. The latter are the results of a

## CHEMICALS SIMULATING EFFECTS OF IONIZING RADIATIONS

complex chain of reactions which can be initiated by fundamentally different events'. This view finds considerable support wherever detailed investigations on the mechanism have been carried out.

*Chromosome fragmentation*—The structural changes in the nucleus produced by the radiomimetic chemicals are qualitatively indistinguishable from those brought about by x-rays. This was observed originally by KOLLER<sup>10</sup> with mustard gas both with the root-meristem of *Allium* and with *Tradescantia* pollen, and subsequently in other materials such as tumour cells. However, distinct differences were revealed between the chemical and physical agents in more detailed studies.

(i) The period of maximum sensitivity of the cell occurs earlier in the mitotic cycle for the chemicals than for x-rays. This was first

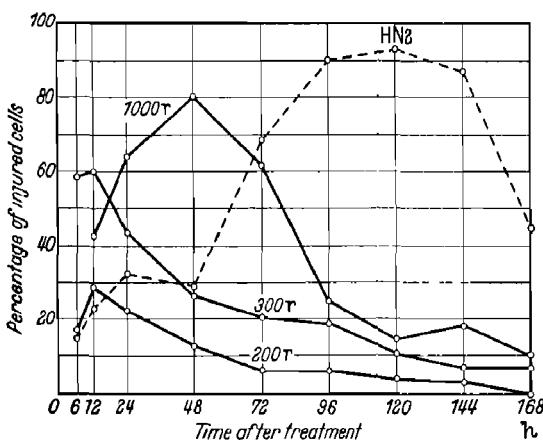


Figure 4. Graph illustrating the difference in the time of max. sensitivity of rat tumour cells to x-rays and to the nitrogen mustard HN2.<sup>37</sup>

demonstrated by KOLLER and CASARINI<sup>37</sup> using rat tumour material and the nitrogen mustard,  $\text{CH}_3\text{N}(\text{CH}_2\text{CH}_2\text{Cl})_2$ , usually referred to as HN2 (see Figures 4 and 5). REVELL<sup>38</sup> showed the same effect with a bis-epoxide in *Vicia* (see Figure 6). The data leaves no doubt that the chemicals act much earlier in the resting stage than the radiations.

(ii) In general, breaks produced by radiations both between different chromosomes and along the same chromosome are distributed at random. In some instances evidence for localization was obtained, but in no case was this very pronounced. The position is quite different with regard to the chemicals. FORD<sup>39</sup> observed that the short chromosomes in *Vicia* were broken much more frequently than the long chromosomes and that certain regions along

X-ray

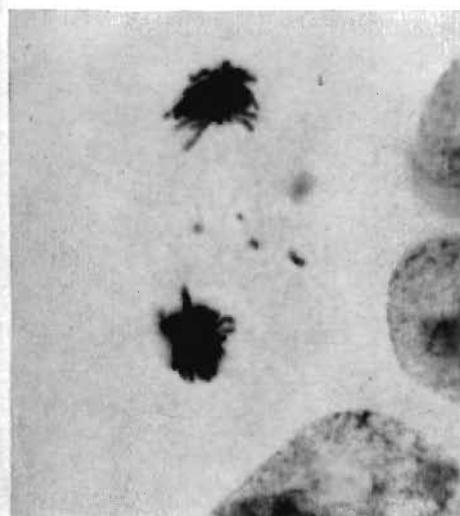
HN2

Time after  
treatment

24h



48h



72h

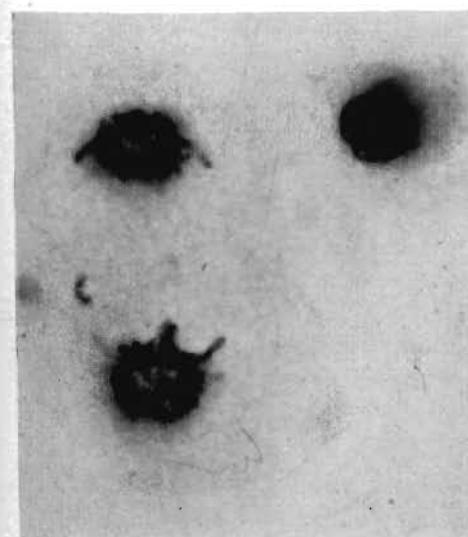


Figure 5. Photo-micrographs illustrating the difference in response of tumour cells to x-rays and the nitrogen mustard, HN2. At a time when the irradiated cells appear nearly normal those treated with HN2 show the max. damage<sup>37</sup>

## BIOLOGICAL ACTION OF THE RADIOMIMETIC SUBSTANCES

the chromosomes were more 'sensitive'. These findings were extended by REVELL<sup>38</sup> and his results are summarized in *Figure 7* which demonstrates clearly the much greater susceptibility of the heterochromatic region to radiomimetic chemicals\*. This observation supports the view that these agents react particularly readily with DNA which is believed to be present in high concentration in the heterochromatin (see p. 179). At present it cannot be decided whether the greater specificity of the chemicals compared with the radiations is due to chemical or physical differences. The chemicals, unlike the free radicals produced by the radiations, have to diffuse through the cytoplasm and the nuclear membrane before they can influence the chromatine or its synthesis. For this reason those chromosomes or part of the chromosomes closest to the nuclear

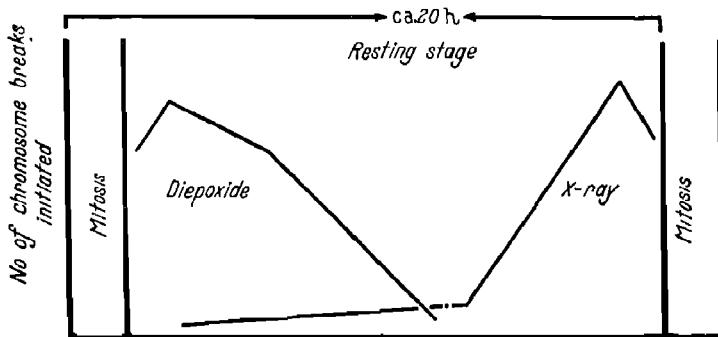


Figure 6. Changes in cell sensitivity in *Vicia faba* to the initiation of chromosome breaks by a bis-epoxide and by x-rays<sup>38</sup>

membrane during the resting stage would be more susceptible to damage by the chemicals.

(iii) The number of chromosome aberrations in *Vicia* produced by a bis-epoxide are a direct function of both the time of treatment with and the concentration of the chemical (e.g.  $2.5 \times 10^{-4} M$  solution for 4 hours is equivalent to  $10^{-3} M$  for 1 hour)<sup>38</sup>. This behaviour is quite different from the breaks produced by radiation where dose rate is as important as total dose.

This evidence† can only be interpreted on the assumption that the primary reactions which induce the series of reactions leading to

\* Other chemicals also show selectivity but the sensitive regions are not the same. For example, on treatment with urethane<sup>40</sup>, ethoxycaffeine and tetramethyl uric acid<sup>41</sup> breaks occur preferentially in the long ('M') chromosome of *Vicia*.

† In addition to the points enumerated above there are also a number of other differences<sup>38</sup> between the radiation and the chemically produced breaks, but these are too technical to be dealt with here.

#### CHEMICALS SIMULATING EFFECTS OF IONIZING RADIATIONS

the observed chromosome abnormality are not the same for the radiomimetic chemicals as for the ionizing radiations.

*Production of gene-mutations*—In many cases (e.g. for *Neurospora*<sup>42</sup> and *Drosophila*<sup>43</sup>) the similarity between the genetic effects produced by radiation and chemical-mutagens is very close. *Table IV* shows

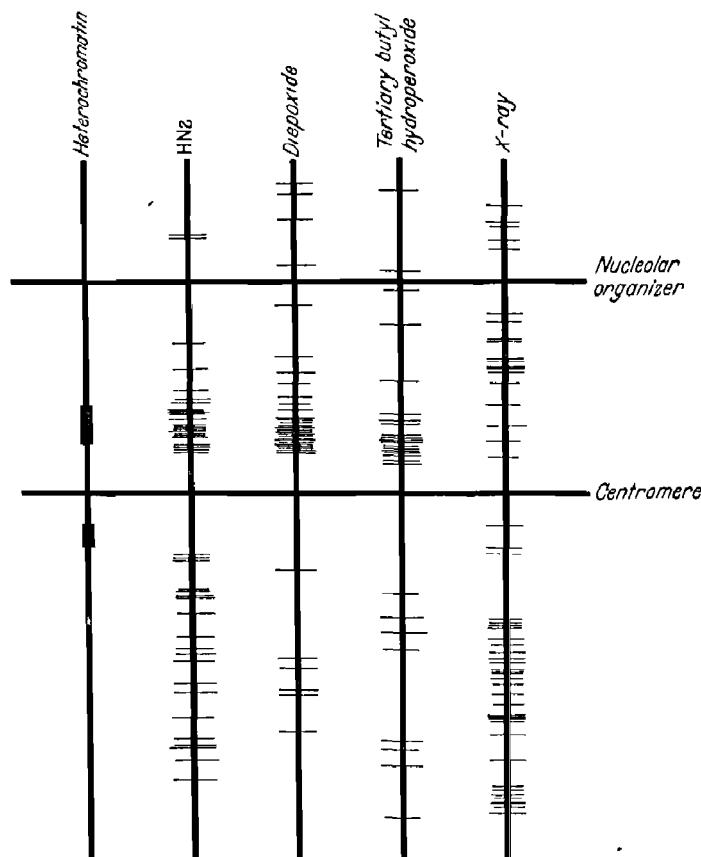


Figure 7. Position of two heterochromatic segments and of a series of breaks induced in the L chromosome of *Vicia faba*

that qualitatively even some non-radiomimetic cell poisons can bring about all the different genetic effects in *Drosophila* which are induced by x-rays. Even more striking are the following experiments which show that the mustards can produce inherited end-effects which are indistinguishable from those produced by ionizing radiations.

## BIOLOGICAL ACTION OF THE RADIOMIMETIC SUBSTANCES

Table IV. Comparison of the Genetic Effects produced in *Drosophila* by x-rays and by different Chemicals<sup>43</sup>

Effect	x-rays	Sulphur mustard	Nitrogen mustard HN2	Formaldehyde	Urethane
Recessive lethals	+	+	+	+	+
Dominant lethals	+	+	+	+	?
Visible mutations	+	+	+	+	+
Minor deficiencies	+	+	+	+	+
Gross deletions.	+	+	+	+	+
Inversions	+	+	?	+	?
Translocations.	+	+	+	+	?
Gynandromorphs	+	+	?	+	?

+ Observed.

? Not observed or only very infrequently observed.

(i) When *E. coli* is irradiated with a heavy dose of x-rays the survivors are very resistant to further irradiation and constitute a new strain. In the same way a nitrogen mustard resistant strain can be produced. BRYSON<sup>44</sup> noted that the mustard resistant strain was also resistant to radiations and *vice versa*. Resistance in different strains is usually a very specific phenomenon and the production of cross-resistance indicates a close similarity between the two toxic agents.

(ii) Infra-red irradiation prior to exposure to x-rays increases a specific type of chromosome aberration in *Drosophila*. Post-treatment with infra-red has no such effect. KAUFMAN *et al.*<sup>45</sup> found exactly the same behaviour when HN2 is used instead of x-rays.

With mutations the evidence for a difference in mechanism is not so strong as in the production of visible chromosome aberrations and the most significant experiment in this respect is probably the observation of AUERBACH<sup>46</sup> that some of the genetic effects of treatment with mustard are delayed. This was deduced from the fact that the number of *Drosophila* with mosaic areas in the body is very much greater, and the area much smaller, after mustard than after x-ray treatment. Mosaics are the result of chromosome changes brought about after the fertilized egg has started to divide. If the aberration occurs at the first division half the body of the fly will be affected—this is the case with x-rays. If it occurs at later cleavage divisions the mosaics will cover a smaller area. Somatic crossing-over, another delayed effect, is also much more frequent with mustard than with x-ray treatment.

## CHEMICALS SIMULATING EFFECTS OF IONIZING RADIATIONS

### CLINICAL APPLICATIONS

The use of ionizing radiations in cancer therapy is not due to any inherent difference in response of normal and malignant cells, but depends on the greater sensitivity of dividing cells. Whatever the difference in mechanism between the radiomimetic agents and radiations may be, the former show no greater selectivity for tumour cells than radiations. None of the many hundreds of nucleotoxic\* substances examined have given any indication of use as chemotherapeutic agents against cancer. A number of the radiomimetic substances have, however, a rather potent action against particular blood cells in the bone-marrow and this makes them useful for the therapy of some of the leukaemias<sup>47</sup>. Moreover, different members of the radiomimetics do not influence the blood picture of the animal in the same way. The mesyloxy compound,



which is marketed under the name of Myleran, affects primarily the granulocyte precursors powerfully at low doses, and this makes it a most valuable chemotherapeutic agent for the control of myeloid leukaemia<sup>48</sup>. This selectivity is, however, not related to malignancy and is merely due to the fact that certain quite normal cells are more vulnerable than others. Certain nitrogen mustards were shown by ELSON and GALTON<sup>49</sup> to have an entirely different effect on the blood picture and to resemble closely the initial x-ray effect.

The nitrogen mustards, particularly di-2-chloroethylmethylamine, HN2, are now in routine use in the treatment of the generalized phases of the lymphomas, especially Hodgkin's disease<sup>49, 50, 63</sup>. Tri-ethylenemelamine is even more potent but is also more toxic and is less widely used. These agents are purely palliative and never eradicate the disease but have real value in their ability to alleviate, for several months, the toxæmic manifestations of the disease, and to relieve pain and other symptoms in circumstances when radiotherapy cannot be used. Urethane is most useful in relieving the pain of multiple myelomatosis, but as with all other chemotherapeutic agents so far studied has no influence on the course of the disease although occasionally the bone lesions recalcify.

\* KOLLER<sup>10</sup> has introduced the useful terminology of nucleotoxic for typical radiomimetic effects which are permanent (*e.g.* chromosome breakage and re-union); cytotoxic effects are initiated by action in the cytoplasm and include the physiological chromosome aberrations (see p. 162). A cytotoxic agent but not a nucleotoxic agent can kill a cell even when it is not dividing (*e.g.* in the resting stage). At high doses the radiomimetics become cytotoxic (*c.f.* footnote p. 191).

## MECHANISM OF ACTION OF RADIOMIMETIC ALKYLATING AGENTS

### MECHANISM OF ACTION OF THE RADIOMIMETIC ALKYLATING AGENTS

Since the cell is most sensitive to the radiomimetics in that part of the resting stage, when Howard and Pelc find that incorporation of phosphorus, supplied by inorganic salts, into DNA is at its maximum (see p. 181) it seems probable that the chemicals interfere with a synthetic process; possibly one connected with the duplication of the chromosomes. For this reason attention has been focussed on the reaction with DNA or its precursors. Physico-chemical studies lend support to this view since DNA reacts much more readily with many of the radiomimetic agents than do most proteins<sup>27</sup>. Interference with SH groups is not indicated since many substances which combine under physiological conditions with SH groups are not radiomimetic<sup>61</sup> and since the radiomimetic agents failed to inactivate a number of —SH enzymes *in vivo*<sup>62</sup>. The recent recognition of the importance of coenzyme A (see *Figure 2*, p. 242) in many synthetic processes would warrant an examination of the possibility that the radiomimetic agents interfere with these enzymatic activities catalysed by this enzyme.

'*Degradation*' of DNA—Preoccupation with the close similarity in the biological lesions produced led to speculation concerning a common chemical mechanism between the radiomimetic agents and ionizing radiations. In the same year, 1946, GJESSING and CHANUTIN<sup>51</sup> found that the viscosity of DNA solutions was decreased by reaction with nitrogen mustard HN<sub>2</sub>, and SPARROW and ROSENFELD<sup>52</sup> obtained the same effect by irradiation with x-rays. TAYLOR, GREENSTEIN and HOLLAENDER<sup>53</sup> subsequently showed that the ionizing radiations broke up the DNA molecule and reduced its molecular weight (see Chapter 4). BUTLER and his colleagues<sup>54</sup> extended these investigations (see *Figure 8*) and concluded that since the viscosity change was of the same character with HN<sub>2</sub> as with x-rays the former also degraded the molecule. This led to the hypothesis that the mustards like x-rays degrade by a free radical mechanism<sup>55</sup>. Chemical experiments designed to test this suggestion failed to provide any support for it<sup>27</sup> and at least one<sup>56</sup> of the postulated mechanisms was shown to be impossible on quantum-mechanical grounds<sup>57</sup>.

ALEXANDER<sup>27, 35</sup> pointed out that a decrease in viscosity of a complex macromolecule does not necessarily imply that the molecule has been degraded and that exactly the same change will result from a change in shape. This was demonstrated experimentally<sup>58</sup> with polymethacrylic acid. On irradiation with x-rays the polymer

### CHEMICALS SIMULATING EFFECTS OF IONIZING RADIATIONS

becomes degraded (see p. 132) while nitrogen mustard, HN<sub>2</sub>, reduced the viscosity by internally crosslinking the molecule, thereby changing its shape. All the available evidence (*cf.*<sup>27</sup>) now points to the conclusion that the initial reaction of the radiomimetic agents with DNA results in a change of shape of the molecule and does not

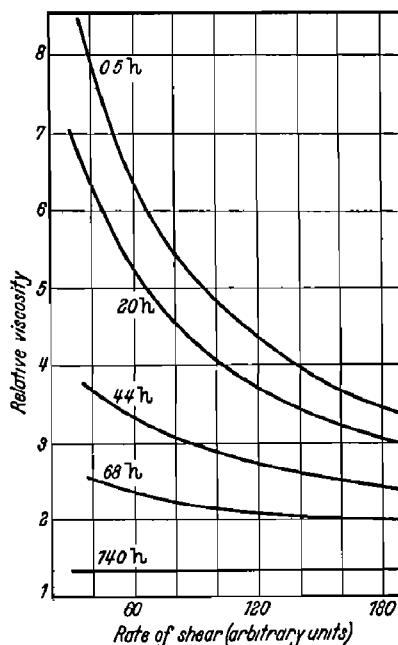
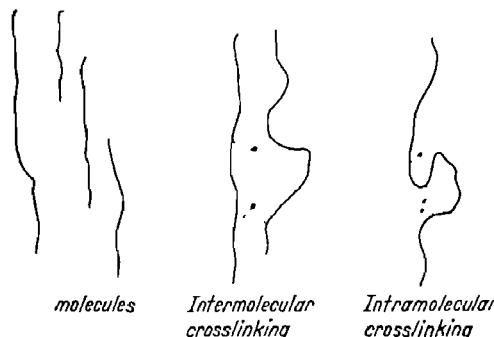


Figure 8. Decrease in viscosity of DNA (0.1 per cent soln) after different times following treatment with HN<sub>2</sub>.<sup>54</sup>

Figure 9. Inter- and intra-molecular crosslinking of macromolecules (..... represents crosslinks)



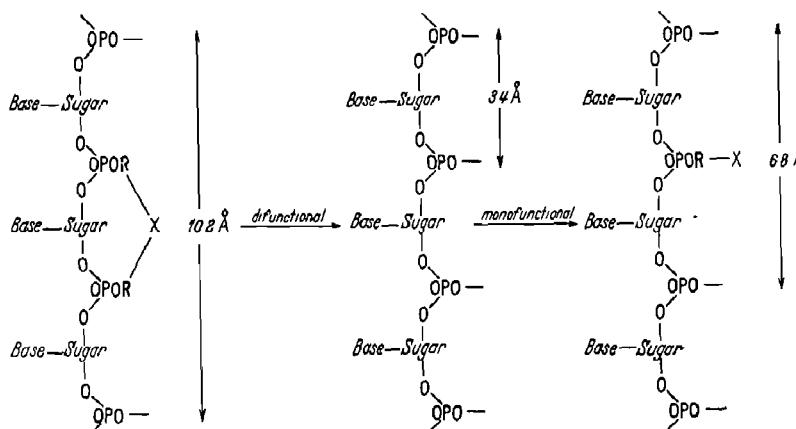
bring about degradation. This is an example on a chemical level of the danger of inferring an identity of mechanism from a similarity in end-effect.

*The crosslinking hypothesis*—Following on the finding that biological activity of the nitrogen mustards, as judged by growth inhibition,

MECHANISM OF ACTION OF RADIOMIMETIC ALKYLATING AGENTS

required two reactive halogenoalkyl groups led to the suggestion that a crosslinking reaction was involved<sup>21, 59</sup>. The extension of this idea to the formation of chromosome abnormalities by crosslinking chromatids is untenable in view of more recent cytological data. In its more general context the theory has many attractive features and covers intra- as well as inter-molecular crosslinking of macromolecules, the latter lead to a decrease in viscosity while the former will bring about an increase (see *Figure 9*). The major difficulty facing the crosslinking hypothesis is the fact that while the presence of two or more alkylating centres per molecule confers high biological activity monofunctional compounds are nevertheless radiomimetic (see p. 194).

ALEXANDER<sup>35</sup> found that the phosphate groups of DNA in dilute



*Figure 10.* Postulated reaction of a bifunctional alkylating agent with DNA<sup>35</sup>

aqueous solution were esterified at similar rates by the corresponding mono-, and poly-functional alkylating agents, though the rates varied from one type of radiomimetic compound to another. It appears unlikely, therefore, that this reaction alone is biologically significant. However, the affinity of DNA for the protamines was reduced much more by prior combination with a polyfunctional than a monofunctional compound. A possible interpretation is that the bifunctional compounds can bring about a change in shape of the DNA molecule, which would affect its interaction with other molecules, more readily than the monofunctional derivatives. The biological action of the radiomimetic agents could be ascribed, on the basis of these experiments, to interference with the normal

## CHEMICALS SIMULATING EFFECTS OF IONIZING RADIATIONS

formation of nucleoprotein; or, if the suggestion of HAUROWITZ<sup>60</sup> is accepted that DNA maintains a 'template protein' in an expanded state during biosynthesis coiling of the DNA would interfere with protein synthesis. ALEXANDER<sup>35</sup> suggested that the reason for the greater effectiveness of the polyfunctional compounds was that it is necessary to react with neighbouring phosphate groups so as to alter an appreciable area of the DNA (see *Figure 10*). Clearly the probability that two monofunctional compounds react with adjacent groups is very small and this may explain why it is necessary to use them at 50 to 100 times the concentration of the corresponding poly-functional compounds. The steric requirement for high biological activity (see footnote p. 193), that the reactive groups within the same molecule should be placed in such a way that they can form a ring, follows directly if reaction with adjacent groups is required.

## REFERENCES

- <sup>1</sup> DUSTIN, P., *Nature, Lond.*, 1947, **159**, 794
- <sup>2</sup> DUSTIN, P., *Bull. Acad. Méd. Belg.*, 1934, **14**, 437
- <sup>3</sup> KENNAWAY, E. L., and HIEGER, I., *Brit. med. J.*, 1930, 1044
- <sup>4</sup> AUERBACH, C. and ROBSON, J. M., *Nature, Lond.*, 1944, **154**, 81
- <sup>5</sup> BACQ, Z. M., *Enzymologia*, 1941, **10**, 48  
BACQ., Z. M., *Bull. Acad. Méd. Belg.*, 1946, **11**, 137
- <sup>6</sup> LOVELESS, A., *Nature, Lond.*, 1951, **167**, 338
- <sup>7</sup> HARRIS, E. B., LAMERTON, L. F., ORD, M. J. and DANIELLI, J. F., *Nature, Lond.*, 1952, **170**, 921
- <sup>7a</sup> DUSTIN, P. Jr., *Rev. Hémat.*, 1950, **5**, 603  
DUSTIN, P. Jr., *C.R. Soc. Biol., Paris*, 1950, **144**, 1297  
GRAMPA, G. and DUSTIN, P. Jr., *Rev. belge Path.*, 1952, **22**, 113
- <sup>7b</sup> BINET, L. and WELLERS, G., *Bull. Soc. Chim. biol., Paris*, 1946, **28**, 751
- <sup>8</sup> OCKHLER, F., *Z. indukt. Abstamm. u. VererbLehre*, 1943, **81**, 313
- <sup>9</sup> OCKHLER, F., *S. B. heidelberg Akad. Wiss.*, 1949, **9**, 373
- <sup>10</sup> KOLLER, P., *Progr. Biophys.*, 1954, **4**, 195
- <sup>11</sup> LOVELESS, A. and ROSS, W. C. J., *Nature, Lond.*, 1950, **166**, 1112
- <sup>12</sup> DICKEY, H. H., CLELAND, G. H. and LOTZ, G., *Proc. Nat. Acad. Sci., Wash.*, 1949, **35**, 581
- <sup>13</sup> HADDOCK, A., HARRIS, R. J. C., KON, G. A. R. and ROE, E. M. F., *Phil. Trans.*, 1948, **241**, 147
- <sup>14</sup> BOYLAND, E., *Biochim. biophys. Acta*, 1950, **4**, 293
- <sup>15</sup> DEMERECK, M., *Brit. J. Cancer*, 1948 **B2**, 114
- <sup>16</sup> DEMERECK, M., BERTANI, G. and FLINT, J., *Amer. Nat.*, 1951, **85**, 119
- <sup>17</sup> CHASE, H. B., *Science*, 1951, **113**, 714
- <sup>18</sup> BOYLAND, E. and SARGENT, S., *Brit. J. Cancer*, 1951, **5**, 433
- <sup>19</sup> LANDING, B. D., GOLDIN, A., NOE, H. A., GOLDBERG, B. and SHAPIRO, D. M., *Cancer*, 1949, **2**, 1055

#### REFERENCES

- <sup>20</sup> SMITH, T. R., JACOBSON, L. O., SPURR, C. L., ALLEN, J. G. and BLOCK, M. H., *Science*, 1948, **107**, 474
- <sup>21</sup> HADDOW, A., 'Newer concepts in the chemistry of growth', *Proc. First nat. Cancer Conf.*, Memphis, 1949, p. 88
- <sup>22</sup> PHILIPS, F. C., *J. Pharmacol.*, 1950, **99**, 281
- <sup>23</sup> HADDOW, A., KON, G. A. R. and ROSS, W. C. J., *Nature, Lond.*, 1948, **162**, 824
- <sup>24</sup> BURCHENAL, J. H. and RILEY, J. B., *Cancer Res.*, 1949, **9**, 553
- <sup>25</sup> ROSS, W. C. J., *J. chem. Soc.*, 1949, 183  
ROSS, W. C. J., *Advances in Cancer Research*, 1953, **1**, 397
- <sup>26</sup> HADDOW, A., *The Physiopathology of Cancer*, New York, Chapter 16, 1954
- <sup>27</sup> ALEXANDER, P., *Advances in Cancer Research*, 1954, **2**, 1
- <sup>28</sup> BIRD, M. J., *J. Genet.*, 1952, **50**, 480  
BIRD, M. J. and FAHMY, O. G., *Proc. roy. Soc.*, 1953, **B140**, 556
- <sup>29</sup> KOLLER, P. C., Report to Ministry of Supply by J. M. Robson, October 1943, London, H.M. Stationery Office  
DARLINGTON, C. D. and KOLLER, P. C., *Heredity*, 1947, **1**, 187
- <sup>30</sup> HENDRY, J. A., HOMER, R. F., ROSE, F. L. and WALPOLE, A. L., *Brit. J. Pharmacol.*, 1951, **6**, 357  
HENDRY, J. A., HOMER, R. F., ROSE, F. L. and WALPOLE, A. L., *ibid.*, 1951, **6**, 235
- <sup>31</sup> BIESELE, J. J., PHILIPS, F. S., THIERSCH, J. B., BURCHENAL, J. H., BUCKLEY, S. M. and STOCK, C. C., *Nature, Lond.*, 1950, **166**, 112
- <sup>32</sup> STEVENS, C. M. and MYLROIE, A., *Biochim. biophys. Acta*, 1952, **8**, 325
- <sup>33</sup> ALEXANDER, P., FOX, M. J., STACEY, K. A. and SMITH, L. F., *Biochem. J.*, 1952, **52**, 177
- <sup>34</sup> ELMORE, D. T., GULLAND, J. M., JORDAN, D. O. and TAYLOR, H. F. W., *Biochem. J.*, 1948, **42**, 308
- <sup>35</sup> ALEXANDER, P., *Nature, Lond.*, 1952, **169**, 226
- <sup>36</sup> OGSTON, A. G., *Trans. Faraday Soc.*, 1948, **44**, 45
- <sup>37</sup> KOLLER, P. C. and CASARINI, A., *Brit. J. Cancer*, 1952, **6**, 173
- <sup>38</sup> REVELL, S. H., *Heredity, Suppl.*, 1953, **6**, 107
- <sup>39</sup> FORD, C. E., 'Chromosome breakage in nitrogen mustard treated *Vicia faba* root tip cells', *Proc. 8th int. Congr. Genetics, Lund*, 1948, p. 570
- <sup>40</sup> DENFEL, J., *Chromosoma*, 1951, **4**, 239
- <sup>41</sup> HIEGER, I., and PULLINGER, B. D., *Recent Advances in Pathology*. 6th edn, Churchill, London, 1953, p. 143
- <sup>42</sup> HOROWITZ, N. H., *Science*, 1946, **104**, 233
- <sup>43</sup> AUERBACH, C., *Cold Spr. Harb. Symp. quant. Biol.*, 1951, **17**, 199
- <sup>44</sup> BRYSON, V., *J. Bact.*, 1948, **56**, 423
- <sup>45</sup> KAUFMAN, B. P., GAY, H. and ROTHBERG, H., *J. exp. Zool.*, 1949, **111**, 415
- <sup>46</sup> AUERBACH, C., *Proc. roy. Soc. Edinb.*, 1946, **62**, 211
- <sup>47</sup> HADDOW, A., *British Surgical Progress*, p. 256, Butterworths, London, 1953
- <sup>48</sup> HADDOW, A. and TIMMIS, G. M. *Lancet* 1953, **1**, 207  
GALTON, D. A. G., *Lancet*, 1953, **1**, 208
- <sup>49</sup> ELSON, L. A., *Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955
- <sup>50</sup> GALTON, D. A. G., *Brit. J. Radiol.*, 1951, **24**, 511

CHEMICALS SIMULATING EFFECTS OF IONIZING RADIATIONS

- <sup>51</sup> GJESSING, E. C. and CHANUTIN, A., *Cancer Res.*, 1946, **6**, 593  
<sup>52</sup> SPARROW, A. H. and ROSENFIELD, F. M., *Science*, 1946, **104**, 245  
<sup>53</sup> TAYLOR, B., GREENSTEIN, J. P. and HOLLOWENDER, A. E., *Arch. Biochem.*, 1948, **16**, 19  
<sup>54</sup> BUTLER, J. A. V., GILBERT, L. A. and SMITH, K. A., *Nature, Lond.*, 1950, **165**, 714  
CONWAY, B. E., GILBERT, L. and BUTLER, J. A. V., *J. chem. Soc.*, 1950, 3421  
<sup>55</sup> BOYLAND, E., *Endeavour*, 1952, **11**, 87.  
<sup>56</sup> BUTLER, J. A. V., *Nature, Lond.*, 1950, **166**, 18  
<sup>57</sup> JENSEN, E. V., *Trans. 5th Conf. Biol. Autoxidants*, 1950, Josiah Macy Jr. Foundation, New York, p. 159  
<sup>58</sup> ALEXANDER, P. and FOX, M., *Nature, Lond.*, 1952, **A169**, 572  
<sup>59</sup> GOLDACRE, R. J., LOVELESS, A. and ROSS, W. C. J., 1949, *Nature, Lond.*, **163**, 667  
<sup>60</sup> HAUROWITZ, F., *The Chemistry and biology of protein*, Academic Press, New York, 1950  
<sup>61</sup> BACQ, Z. M., 'Travaux récent sur les toxiques de guerre,' *Actualités Biochimiques*, Desoer, Liège, et Masson, Paris, 1947  
<sup>62</sup> NEEDHAM, D. M., *Biochem. Soc. Symposia*, 1948, **2**, 16  
PETERS, R. A., *Nature, Lond.*, 1947, **159**, 149  
<sup>63</sup> BACQ, Z. M., FIRKET, J. and HERVE, A., *Bull. Acad. Méd. Belg.*, 1947, 6th Ser., **12**, 295-336  
<sup>64</sup> TIMMIS, G. M., *Brit. Emp. Cancer Campgn Rep.* 1951, **28**, 59

## THE OXYGEN EFFECT IN RADIobiology

THE importance of the oxygen pressure within irradiated cells, tissues or organisms has received great emphasis in current\* radio-biological studies. The presence of oxygen in irradiated water and aqueous solutions changes the nature of the free radicals arising from the ionization of water, and therefore gives rise to radiochemical reactions which differ from those which occur in the absence of oxygen (see p. 104).

In the radiochemistry of aqueous solutions the effect of oxygen is much greater for  $\text{x}$ - or  $\gamma$ -rays than for  $\alpha$ -particles or neutrons which give rise to tracks containing high concentrations of radicals. If a reaction, whether chemical or biological, produced by  $\text{x}$ - or  $\gamma$ -rays is diminished by the exclusion of oxygen then indirect action, through the mediation of free radicals is indicated although the reverse does not apply. Oxygen may also influence a reaction by adding on to a molecule damaged by either direct or indirect action to give rise to a product which differs from that produced under anoxic conditions. This is a possible mechanism by which oxygen could also enhance primary effects produced by direct action.

In biology the oxygen effect is generally characterized by the following features : (i) In the absence of oxygen or at reduced oxygen pressure the effects of  $\text{x}$ - or  $\gamma$ -rays are diminished but not abolished. (ii) The oxygen has to be present *during* the irradiation and exposure to oxygen before or after the irradiation does not influence the process. (iii) The effects of neutrons, and especially of  $\alpha$ -particles, are much less sensitive to the presence of oxygen†.

The oxygen effect is almost invariably observed ; this is one of the strongest pieces of evidence for the view that *indirect action* plays a part in producing biological lesions. Whether the material studied is a mammal, a bacterium, a yeast, a tissue culture, an insect, or a

\* Apparently, Holthusen in 1921 was the first to observe that organisms became more radiation resistant when the oxygen pressure was lowered<sup>2</sup>; this fact was rediscovered by CRABTREE and CRAMER in 1933<sup>3</sup>. But it was necessary to wait until 1945 before the biologists, having learned of the importance of oxygen in radiation chemistry, investigated the oxygen effect in detail.

† Similarly, the effect of chemical protectors is (i) much more marked for  $\text{x}$ - and  $\gamma$ -rays than for neutrons and  $\alpha$ -particles, and (ii) much more evident in the presence of oxygen.

grain of pollen, and whether the effect observed is the frequency of mutations, the rupture of chromosomes, growth, the death rate in a group, or some biochemical effect, the oxygen effect is always to be found. A monograph might be devoted to it, but it will be enough to quote some definite examples and a few sound detailed publications to justify the assertion that the oxygen effect is one of the laws of general radiobiology\*.

#### THE OXYGEN EFFECT IN MICROBIOLOGY

From the technical point of view, micro-organisms or single cells which adapt themselves equally well to aerobic and anaerobic conditions form the material of choice. This was well understood by HOLLÄENDER and his collaborators in a remarkable series of studies on a strain of *Escherichia coli*<sup>11-13</sup>.

*Figure 1* gives the result of a typical experiment by these authors. It shows the survival curves of *E. coli* cultivated aerobically and irradiated in a solution saturated with oxygen (*I*), cultivated anaerobically and irradiated in a solution saturated with nitrogen (*II*), cultivated aerobically and irradiated under nitrogen (*III*), and cultivated anaerobically and irradiated under oxygen (*IV*).

Several obvious conclusions can be drawn: the difference in sensitivity between bacilli cultivated and irradiated in air and similar bacilli cultivated and irradiated in the absence of oxygen is so great that 10 times as much energy is needed to inactivate the same proportion of anaerobic as of aerobic bacilli, and, with a given dose of x-rays, the proportion of aerobic bacteria inactivated is 100,000 times greater. Other inert gases may be used instead of nitrogen without changing the result and the important factor is the presence of oxygen. Similarly, the sensitivity of *E. coli* cultivated anaerobically is increased as soon as oxygen is introduced.

*The oxygen, therefore, acts essentially during the irradiation;* it matters little if the cultures are kept afterwards in aerobic or anaerobic conditions. These facts have been confirmed in very diverse biological systems. *Table I* sums up the observations of Giles and Riley on the microspores of *Tradescantia paludosa*. Oxygen acts on the fundamental primary radiation effect, *i.e.* on the lesion (probably indetectable), as soon as irradiation is ended.

Oxygen by itself is not devoid of action<sup>21</sup>. If pollen or microspores of *Tradescantia* are exposed to partial oxygen tensions higher than that of air, chromosome breakage is observed. In fact, the

---

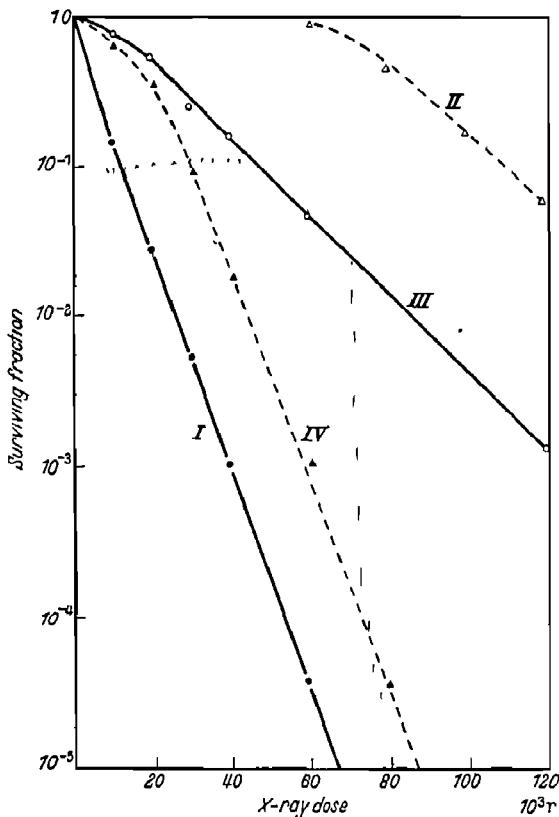
\* As would be expected on chemical grounds the biological reactions to u.v. and the mustards are independent of oxygen.

### THE OXYGEN EFFECT IN MICROBIOLOGY

*Table I. Effects of Conditions during Irradiation and Treatment after Irradiation on the Chromosomes of Microspores of Tradescantia. Summary by HOLLAENDER et al., from GILES and RILEY (1950)*

Condition during irradiation (300 r at 300 r/min)	Condition after irradiation	Number per cell	
		Interchanges	Interstitial deletions
Vacuum	Vacuum	0.12	0.11
Vacuum	Oxygen	0.09	0.10
Oxygen	Oxygen	0.70	0.83
Oxygen	Vacuum	0.72	0.85

alterations in the chromosomes produced by oxygen appear to be identical with those caused by ionizing radiations. As many



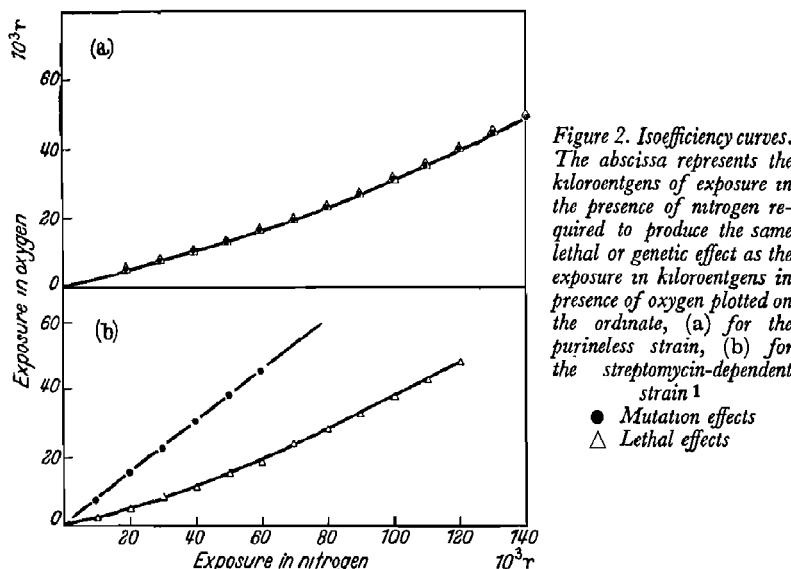
*Figure 1. Comparative sensitivity of aerobic and anaerobic cells irradiated in high and low oxygen tensions. E. coli irradiated in buffer soln with 250 kV X-rays: I Aerobic broth cells irradiated in oxygen saturated buffer; II Anaerobic glucose cells irradiated in nitrogen saturated buffer; III Aerobic broth cells irradiated in nitrogen saturated buffer; IV Anaerobic glucose cells irradiated in oxygen saturated buffer*

changes in the chromosomes are observed if grains of pollen are kept for an hour in pure oxygen as after a dose of 1200 r of X-rays.

## THE OXYGEN EFFECT IN RADIobiOLOGY

Normal air (20 per cent oxygen) at high pressure is as efficient as atmospheres enriched with oxygen; the important point is the partial pressure of oxygen<sup>21</sup>. It is a curious fact that the best protectors against x-rays, i.e. cysteamine and cysteine (see p. 293), are also the best protectors of mammals against oxygen poisoning and disturbances caused by air at high pressures\*.

ANDERSON<sup>1</sup> compared the changes in mortality and in the frequency of mutations due to x-rays in two strains of *E. coli* in the



presence and absence of oxygen. For the mortality of one streptomycin-dependent strain he found a dose-reduction value of 2.5. For a second, purineless strain, a biochemical mutant which requires adenine as a growth substance, the value is 3, that is to say three times less energy in the form of x-rays is needed to obtain the same mortality and the same frequency of mutation (back mutation, restoration of the ability to form purines) when oxygen is present as when it is absent. Therefore in this purineless biochemical mutant, oxygen modifies a genetic and a somatic phenomenon quantitatively in the same way.

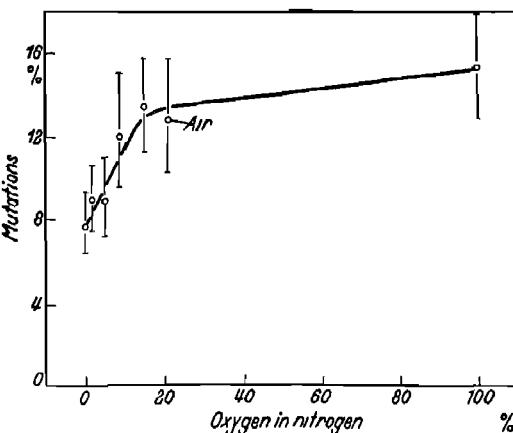
This is not true of the streptomycin-dependent strain (Figure 2), in which the number of mutations caused by x-rays is but slightly in-

\* In this connection it is interesting to note that ABEL<sup>24</sup> has suggested that when oxygen is dissolved in water radicals are formed in very low concentrations by the following reaction  $\text{H}_2\text{O} + \text{O}_2 \rightarrow \text{OH}^\cdot + \text{HO}_2^\cdot$ . These are the same radicals as are responsible for the indirect action of ionizing radiation in aqueous systems.

#### THE OXYGEN EFFECT IN MICROBIOLOGY

creased in the presence of oxygen. To many geneticists these experiments would seem to prove that microbiologists misuse the term 'mutation', that the genetics of bacteria are in their infancy, and that an attempt is being made to relate fundamentally different facts. In a well-tested material, *Drosophila*, the percentage of the least disputable mutations (sex-linked lethal mutations) increases proportionally with the oxygen concentration during irradiation of between 0 and 15 per cent (Figure 3), the zone in which the oxygen effect is noted in the depolymerization of polymethacrylic acid (see p. 106), the slope is also the same. Mutations occur at a certain frequency even in the complete absence of oxygen. These are caused either

Figure 3. Relationship between oxygen concn and x-ray-induced mutation rate for sex-linked lethals in *D. melanogaster* (400 r)



by the direct action of x-rays or by the free radicals formed by the ionization of water in the absence of oxygen

HOLLAENDER (1951) discusses the interpretation of these experiments and shows that, in all probability, oxygen does not act by altering the 'sensitivity' of the cells, but rather by increasing the quantity mutagenic substances produced during irradiation.

It need not necessarily be concluded from this that the presence of oxygen after irradiation is of no consequence. If oxygen is necessary for metabolism (as in all aerobic organisms), the possible restorative mechanisms will to a great extent be inhibited if the irradiated organism is kept in an atmosphere devoid of oxygen. However, no conclusive experiments have been carried out to test this point in the case of irradiation with x-rays.

#### THE OXYGEN EFFECT ON MAMMALS

As far as is known the living material most sensitive to the oxygen effect is the isolated lymph gland of the rat, cultivated *in vitro* by

### THE OXYGEN EFFECT IN RADIobiOLOGY

TROWELL<sup>16, 17</sup>. The small lymphocytes in these glands are the most radiosensitive, though they no longer divide and have reached the end of their mitotic career\*.

The dose needed to produce pyknosis in half the cells in pure oxygen is 275 r *in vitro* (150 r *in vivo*). To produce the same effect in an atmosphere of pure nitrogen, a dose of 3300 r or 12 times as much energy, is needed. The effect of oxygen is greater on this tissue than on plants and insects, showing a linear increase with an oxygen concentration of the atmosphere of 0 to 100 per cent (or 0 to 2 per cent in the culture medium), whereas in *Tradescantia* or *Drosophila* the oxygen effect does not increase appreciably at concentrations above 20 per cent oxygen (cf. Figures 4 and 3).

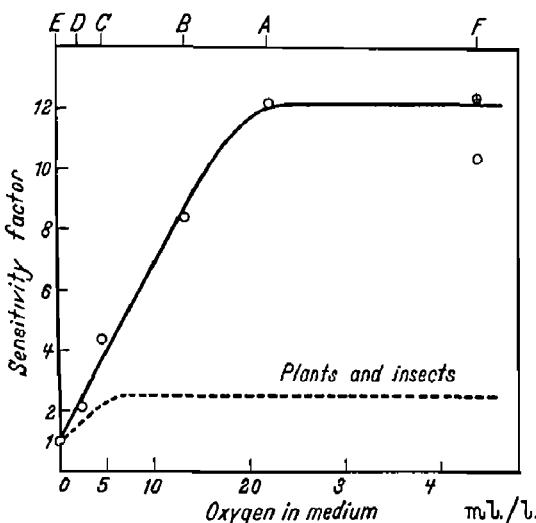


Figure 4. Influence of oxygen on damage to lymph nodes exposed to X-radiation in vitro

Much greater caution is needed in the interpretation of the oxygen effect when whole vertebrates, and especially mammals, are considered.

The first question is that asked at the Aarhus symposium in 1953, where it remained unanswered: *What is the partial pressure of oxygen in the tissues and cells of a mammal?* CAMPBELL<sup>5</sup> reviewed this problem in 1931, and concluded that the tension in the tissues is the same as that in the venous blood or lymph, *i.e.* 20 to 40 mm mercury. MILLIKAN<sup>15</sup> discovered an intracellular indicator of

---

\* Bergonié-Tribondeau's law does not apply in this case. It would be desirable to find out (i) what is the biochemical factor in this radiosensitivity, and (ii) whether other lymphoid tissues behave in the same way.

#### THE OXYGEN EFFECT ON MAMMALS

oxygen tension, myoglobin (or myohaemoglobin, MHb\*), and was able to measure instantaneously the oxygen concentration of the MHb of the cells of the soleus muscle of cats with intact blood supply and innervation. In the resting muscle the MHb is saturated, that is to say referring to the dissociation curve published by Hill, the oxygen tension within the muscle cell is at least 20 mm mercury as Campbell foresaw.

As soon as the muscle is tetanized, *i.e.* as soon as it consumes oxygen, the degree of saturation of the MHb falls abruptly, but it is completely restored in about 10 seconds when stimulation is stopped if the blood supply is normal, that is, if the artery has not been clamped. It may therefore be assumed that mammalian muscle works at an oxygen tension of 20 to 25 mm mercury. This does not mean, of course, that the same oxygen tension exists in the cells of other tissues (*e.g.* skin), where the blood supply may be greatly reduced by cold or by other conditions.

Very probably the oxygen tension in the deep organs which are as well supplied with blood as the muscles (liver, heart, kidney, spleen, central nervous system) is the same as in resting muscle. In organs or accumulations of cells (such as abscesses) which have little or no blood supply the oxygen tension is, of course, lower, and this may play a part in the radiosensitivity of tumour cells when, as sometimes happens, the vascularization of the tumour is very uneven.

There is a second point which, so far as we know, has not yet been considered, and which seems of fundamental importance. When a mammal is deprived of oxygen a number of reflex reactions takes place; in particular, there is intense stimulation of the sympathetic system, which liberates considerable quantities of adrenaline and noradrenaline from the adrenal medulla, and also at the endings of the adrenergic nerves. Now these two hormones, being phenolic amines are good protectors against x-rays. This has been demonstrated for noradrenaline in mice† by BACQ and HERVE<sup>4</sup> and for adrenaline in rats‡ by J. L. GRAY *et al.*<sup>9</sup>. The attempt to explain the protective effect of adrenaline as a result of temporary tissue anoxia, by J. L. Gray *et al.*, must be rejected on physiological grounds since at the moment when they begin irradiation, 5 minutes after the injection of adrenaline, the peak of the hypertensive effect has been passed. Adrenaline increases the cardiac output and oxygen consumption, and is a powerful vasodilator in many parts of the

\* Myoglobin differs from haemoglobin chiefly by its much greater affinity for oxygen and by its hyperbolic dissociation curve.

† 0.06 to 0.11 mg noradrenaline protects 5 to 8 mice out of 10 against 700 r.

‡ 0.2 mg adrenaline leads to the survival of 20 out of 34 rats whereas only 8 out of 34 controls survive.

#### THE OXYGEN EFFECT IN RADIobiOLOGY

body. It cannot be important that it causes slight anoxia in the skin. To cause true local anoxia lasting at least as long as the irradiation a *local* injection of a fairly high concentration ( $1:10^5$ ) of adrenaline is needed. The most likely explanation is that adrenaline and noradrenaline, like other aromatic amines which *do not cause vasoconstriction* protect by competing for the free radicals arising from the ionization of water (see p. 312). Moreover, the injection of a large dose of adrenaline (not of noradrenaline) causes ACTH secretion by the pituitary, and the hormones of the adrenal cortex come into play.

In mammals, cold also considerably diminishes radiosensitivity in young mice (LACASSAGNE<sup>14</sup>) and in adult rats (HAJDUKOVIC *et al.*<sup>10</sup>), provided irradiation is carried out in deeply-chilled animals, that is, if the cold stimulus has been applied *immediately before* irradiation. The reason for this radioresistance too is open to discussion. There are no records of exact experiments to show whether it is due to a lowering of metabolism, or to a possible anoxia resulting from cold. It is difficult enough to know exactly what happens in hibernating mammals which are stabilized at a low internal temperature<sup>22</sup>.

The fact is, that when a mammal is made anoxic or severely chilled, complex reactions are produced. Micro-organisms or plant cells are more suitable for *quantitative* studies of the oxygen effect. If rats are subjected to an atmosphere containing only 5 per cent of oxygen instead of 20 per cent (the concentration in normal air) their sensitivity to radiations is approximately halved. In order to obtain the same mortality curve among anoxic rats as among controls the dose of x-rays must be approximately doubled; with mice, the protection obtained is smaller<sup>6</sup>.

#### PRACTICAL IMPORTANCE OF THE OXYGEN EFFECT

The oxygen effect is of the highest importance to radiotherapists<sup>20</sup>, for cancer cells react in the same way as any other living material. *Figure 5* shows, firstly, that the ascites cells (of Ehrlich's tumour) show fewer chromosome changes if irradiation with x-rays is carried out in a nitrogen atmosphere, a dose four times as large being needed in a nitrogen atmosphere to produce the same percentage of abnormalities as in an oxygen atmosphere; and secondly, that the effect of neutrons is very little increased by the presence of oxygen.

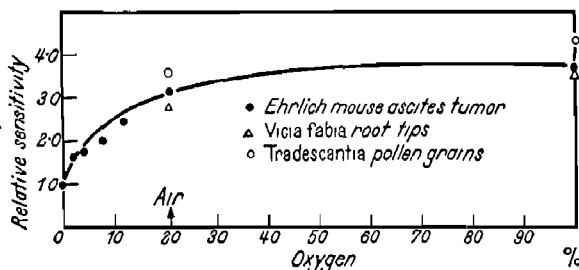
Whether this property of oxygen can be used to increase the effect of x-rays on tumours without increasing their effect on healthy tissues is worth discussing. Supposing firstly that the tumour is

#### PRACTICAL IMPORTANCE OF THE OXYGEN EFFECT

partly or wholly less-well vascularized than healthy tissue, so that the oxygen pressure in the cancer cells is, say, 10 mm mercury, i.e. less than normal (25 mm), and secondly that the oxygen effect in the healthy tissues is already at a maximum, with a pressure of 25 mm. Then, if the oxygen pressure is increased by 15 mm, the effect of x-rays will be increased only in the tumour.

If, on the other hand, all mammalian tissues responded to oxygen in the same way as isolated rat lymph glands (*Figure 4*), this differential effect could not show itself. In Trowell's experiment the reaction of isolated lymph glands to x-rays increased in a straight line up to an oxygen concentration of 20 ml per litre in the culture fluid, and this concentration is reached when the fluid is in equilibrium with pure oxygen at 760 mm mercury. It is true that in this experiment the tissue is not vascularized, and it may be assumed, in

*Figure 5. Dependence of x-ray sensitivity of mouse ascites tumour cells on oxygen tension*



agreement with GRAY<sup>18</sup> that the oxygen tension falls very steeply from the surface to the centre of the ganglion, which must be in an anaerobic state at the oxygen pressure of the atmosphere. The curve might be quite different if a vascularized gland could be used, in which all the cells would live at about the same oxygen tension. It is therefore essential to study the oxygen effect in vascularized mammalian organs quantitatively as fully as is technically possible. It is easy to produce anoxia of the skin: local compression or vasoconstriction due to cold will certainly result in a diminution of the cutaneous lesions when it is desired to irradiate deep-seated tissues.

Conversely, it might be possible to improve the blood supply of poorly irrigated tumours by local injections of vasodilators. The most radical solution, however, supposing the two conditions mentioned above are fulfilled, would be to irradiate the patient in a compression chamber like those used for workers under water or in tunnels. Scott reported a positive experiment of this kind in mice at the Aarhus symposium (1953), see reference 20.

## THE OXYGEN EFFECT IN RADIobiOLOGY

### THE OXYGEN EFFECT IN VIRUSES

Only relatively little data is available concerning the effect of oxygen on the inactivation of viruses. Most studies with viruses have been made under conditions where the direct effect predominates and where no oxygen effect is found. This provides the strongest evidence for the view that the oxygen effect observed in other systems is the result of enhancing the potency of the free radicals responsible for indirect action. ALPER<sup>23</sup> has made a most detailed study of the indirect inactivation of bacteriophage and has shown that the amount of inactivation produced increases with time after the irradiation. Paradoxically she finds that the presence of oxygen *decreases* the initial effect by increasing the after-effect. A negative oxygen effect is therefore found if the activity is measured immediately after irradiation, but a positive oxygen effect will be found if the assay is made after a time-interval of a day or more. ALPER<sup>23</sup> has shown that this progressive inactivation is due to the reaction of the virus, once it has been sensitized by irradiation, with hydrogen peroxide produced by x-rays on aerated water (see p. 149). As the presence of oxygen promotes the formation of hydrogen peroxide (see p. 107) the reaction as a whole seems to be influenced by oxygen. The irradiated phage becomes hyper-sensitive not only to hydrogen peroxide but also to ascorbic acid<sup>22</sup>.

The immediate effect, which is decreased by the presence of oxygen, is increased by the presence of hydrogen gas and this finding led Alper to conclude that the radicals responsible for immediate inactivation act as reducing and not as oxidizing agents. The whole position is very confused since the results with phage appear to differ from those obtained in the degradation by x-rays of DNA in solution (see p. 147). It should, however, be noted that EPHRUSSI-TAYLOR and LATARJET<sup>25</sup> find that the inactivation by indirect action of the pneumococcus transforming factor which consists only of DNA (see p. 177) is not influenced by oxygen. The absence of an oxygen effect in these highly specialized systems although of great interest does not invalidate the conclusions reached from observations made with all living systems concerning the fundamental importance of oxygen in radiobiology.

### REFERENCES

- <sup>1</sup> ANDERSON, E. H., *Proc. nat. Acad. Sci.*, 1951, **37**, 340
- <sup>2</sup> HOLTHUSEN H., *Pflüger's Arch.*, 1921, **187**, 1
- <sup>3</sup> CRABTREE, H. G. and CRAMER, W., *Proc. roy. Soc.*, 1933, **B113**, 226, 238
- <sup>4</sup> BACQ, Z. M. and HERVE, A., *J. suisse Méd.*, 1952, **82**, 160

#### REFERENCES

- <sup>5</sup> CAMPBELL, J. A., *Physiol. Rev.*, 1931, **11**, 1
- <sup>6</sup> DOWDY, A. H., BENNET, L. R. and CHASTAIN S. M., *Radiology*, 1950, **55**, 879
- <sup>7</sup> GILES, N. H. and RILEY, H. P., *Proc. nat. Acad. Sci.*, 1950, **36**, 337
- <sup>8</sup> GRAY, L. H., 'Symposium on chromosome breakage', Suppl., *Heredity*, 1953, **6**
- <sup>9</sup> GRAY, J. L., MOULDEN, E. J., TEW, J. T. and JENSEN, H., *Proc. Soc. exp. Biol. Med.*, 1952, **79**, 384
- <sup>10</sup> HAJDUKOVIC, S., HERVE, A., and VIDOVIC, V., *Experientia*, 1954, **10**, 343
- <sup>11</sup> HOPPENBLUM, A., STAPLETON, G. E. and MARTIN, F. L., *Nature, Lond.*, 1951, **167**, 103
- <sup>12</sup> HOPPENBLUM, A., BAKER, W. K. and ANDERSON, E. H., *Cold Spr. Harb. Symp. quant. Biol.*, 1951, **16**
- <sup>13</sup> HOPPENBLUM, A., STAPLETON, G. E. and BURNETT, W. T., *Ciba Foundn. Conf., London*, 1951
- <sup>14</sup> LACASSAGNE, A., *C.R. Acad. Sci., Paris*, 1942, **215**, 231
- <sup>15</sup> MILLIKAN, G. A., *Physiol. Rev.*, 1939, **19**, 503
- <sup>16</sup> TROWELL, O. A., *Brit. J. Radiol.*, 1953, **26**, 302
- <sup>17</sup> TROWELL, O. A., *J. Path. Bact.*, 1952, **64**, 687
- <sup>18</sup> GRAY, L. H., *Radiation Res.*, 1954, **1**, 180
- <sup>19</sup> HILL, R., *Proc. roy. Soc.*, 1936, **B120**, 472
- <sup>20</sup> GRAY, L. H., CONGER, A. D., EBERT, M., HORNSEY, S. and SCOTT, O. C. A., *Brit. J. Radiol.*, 1953, **26**, 638
- <sup>21</sup> CONGER, A. D. and FAIRCHILD, L. M., *Proc. nat. Acad. Sci.*, 1952, **38**, 289
- <sup>22</sup> KAYSER, C., *Ann. Biol.*, 1953, **29**, 109
- <sup>23</sup> ALPER, T., *Brit. J. Radiol.*, 1954, **27**, 40  
*Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955
- <sup>24</sup> ABEL, E., *Mh. Chem.*, 1951, **82**, 39
- <sup>25</sup> EPHRUSSI-TAYLOR, H. and LATARJET, R., *Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955

## COMPARATIVE RADIOSENSITIVITY OF LIVING ORGANISMS

EVERY degree of radiosensitivity, ranging from 0·01 r, required to modify the growth of the fungus *Phycomyces blakesleeanus*<sup>3</sup>, to  $3 \times 10^5$  r, to kill infusoria, is to be found among living organisms. Tables I and II summarize much of what is known at present. The ideal would be to have a complete curve of survival for each animal, ranging from non-fatal doses to doses which kill during irradiation, similar to the curves drawn for mice by BONET-MAURY and PATTI<sup>1</sup> and by RAJEWSKY<sup>2</sup>, instead of only the LD 50/30 days\* shown in

*Table I. Lethal Dose for 50 per cent of various Animals in 30 days (after RUGH<sup>6</sup> and TOBIAS)*

<i>Organism</i>	<i>Radiation</i>	<i>Dose</i>
<i>Guinea-pig</i>	x-rays	175 to 409 r
<i>Pig</i>	x-rays	275 r
<i>Dog</i>	x-rays	300 to 430 r
<i>Goat</i>	x-rays	350 r
<i>Monkey</i>	x-rays	500 r
<i>Man</i>	x-rays	400 to 500 r
<i>Mouse</i>	x-rays	400 to 650 r
	Fast neutrons	54 n
	$\alpha$ -rays (radon)	14 $\mu$ c/g
	$\beta$ -rays	250 $\mu$ c/g
<i>Rat</i>	x-rays	590 to 970 r
<i>Rabbit</i>	x-rays	750 to 825 r
<i>Hamster</i>	x-rays	725 r
<i>Fowl</i>	x-rays	1000 r
<i>Goldfish</i>	x-rays	670 r
<i>Frog</i>	x-rays	700 r
<i>Newt</i>	x-rays	3000 r
<i>Tortoise</i>	x-rays	1500 r
<i>Snail</i>	x-rays	8000 to 20,000 r
<i>Escherichia coli</i>	x-rays	5600 r
<i>Yeast</i>	x-rays	30,000 r
<i>Amoeba</i>	x-rays	100,000 r
<i>B. mesentericus</i>	x-rays	150,000 r
<i>Colpidium, Paramoecium</i>	x-rays	300,000 r
<i>and Infusoria</i>		350,000 r

\* The dose of irradiation which kills 50 per cent of the animals within 30 days.

## COMPARATIVE RADIOSensitivity OF LIVING ORGANISMS

Table II. Effects of  $\alpha$ -radiation on certain Invertebrates (PEARSON)

Phylum	Genus	Dose (kiloroentgens)	Effect
Ctenophora	Mnemiopsis	1.2 2.4 4.8 to 16.8	Shrinkage, loss of turgidity Partial disintegration Complete disintegration of all specimens
Annelida	Enchytraea	90 in 3 doses	No apparent effects
Mollusca	Radix japonica (adult)	2	LD <sub>50</sub> at 80 days
	Radix japonica (young)	12	LD <sub>50</sub> at 20 days
	Radix japonica (eggs)	1	Reduced activity
		7.2 (estimated)	Prevented formation of all embryos
		8.8 (estimated)	Prevented formation of all veligers
		10.4 (estimated)	Prevented formation of all trochophores
	Thais (adult)	17	LD <sub>50</sub> at 80 days
		20	LD <sub>50</sub> at 5 days
Arthropoda	amphipod (adult)	0.6	LD <sub>50</sub> at 80 days
	amphipod (young)	0.56	LD <sub>50</sub> at 10 days
	Artemia (young 'dry')	93	LD <sub>50</sub> at 5 days
	Artemia (young 'wet')	50	LD <sub>50</sub> at 10 days
		80	LD <sub>50</sub> at 5 days
		20	LD <sub>50</sub> at 10 days
		10	Reduced size after 5 days
	Daphnia magna	6.5	Killed all individuals
	Corethra (Chaor- borus) larvae	0.5 to 3	Midgut cells loosened
		5 to 10	Degeneration of pericardial cells
		> 10 and < 25	LD <sub>50</sub> at 30 days

Table I. The figures in Tables I and II are, of course, only approximate. It is well known that different pure strains of mice or rats show different degrees of radiosensitivity, that slight variations exist even within a single pure strain, and that the physical conditions of irradiation and the methods of measuring doses differ in various schools. Results obtained by different authors are therefore seldom comparable, but certain conclusions can be drawn:

(i) Among the vertebrates, mammals are more sensitive to radiations than birds, fishes, amphibians or reptiles\*. It is easy to understand the difference between mammals and cold-blooded vertebrates, because the latter live at lower temperatures, and in frogs the rate of development of radiation lesions increases with the

\* The amphibia have the advantage that their embryology is well known and that they can live at various temperatures, and are thus very suitable for certain experiments<sup>6-8</sup>.

COMPARATIVE RADIOSENSITIVITY OF LIVING ORGANISMS

temperature (see p. 183). In birds, however, the body temperature and metabolism are higher than in mammals. Work done with pigeons shows that these birds are 5 times less sensitive to radiations than adult rats, and that in them the liver and kidneys are as much affected as the intestines, spleen, bone-marrow and sex glands, which is not true of mammals<sup>23</sup>. There have also been a few studies in fowls. It would be very useful to study other birds as well.

(ii) Unicellular organisms are generally very resistant to radiations (*Table I*), but there is at least one exception<sup>3</sup>, *Phycomyces blakesleeanus*, which reacts to a dose as low as 0.01 r.

Micro-organisms are being increasingly studied as they offer great technical advantages, e.g. in the study of chemical protection and restoration after irradiation. Hollaender, Latarjet, Tobias, and many others are using strains of *E. coli*, lysogenic bacteria, and yeasts respectively (see pp. 59–68) for experimental convenience. The radioresistance of the ciliated Infusoria of the family Ophryoglenidae varies according to the species (*Table III*), the stage of

*Table III. Parallelism between Resistance to Cyanides and to x-rays in Ophryoglenidae (therontes)<sup>16</sup>*

<i>Species</i>	Ophryoglenida pectans	Deltopylum rhabdoïdes	Ophryoglenida atra	Ophryoglenida mucifera
<i>Dose of x-rays lethal in 10 to 15 min in Kr.</i>	600	750	900	1050
<i>Max. well tolerated concn of NaCN .</i>	0.03%	0.05%	0.05%	0.2%

development and the state of nutrition. Starving ‘therontes’ are always the most sensitive to radiations. The higher the resistance to radiations, the higher is also the resistance to cyanide (BACQ, MUGARD and HERVE<sup>16</sup>).

(iii) Insects seem to be rather resistant but less so than unicellular organisms. The several advantages offered by insects in radio-biological investigation have not yet been exploited. Many insects are resistant to cyanide, and can live anaerobically for a long time. Attention has been paid too exclusively to *Drosophila*, the geneticists’ first choice. It is known that the adult *Drosophila* is much more resistant than the young stages. For 3-hour eggs the LD<sub>50</sub> (the dose which kills half the animals) is 200 r, for 4-hour eggs it is 500 r, for 7½-hour eggs it is 810 r, and for pupae it is 2800 r. Adults are

#### COMPARATIVE RADIOSENSITIVITY OF LIVING ORGANISMS

resistant to 64,000 r in the form of  $\gamma$ -rays from  $^{60}\text{Co}$ , but are sterilized; eggs laid after irradiation with 32,000 r or 16,000 r are not viable. A dose of 8000 r allows the laying of many eggs after 4 days' inhibition, but few of these eggs develop<sup>10</sup>. SULLIVAN and GROSCH<sup>9</sup> have described a significant increase in the length of life of a parasitic wasp (*Habrobracon*) after irradiation with doses up to 180,000 r; females are said to be sterilized by doses of about 5000 r.

Some authors do not hesitate to recommend the use of ionizing radiation ( $^{60}\text{Co}$  or the waste products of atomic factories) as insecticides for some insects\* which consume large quantities of stored produce, or attack wool, clothing or wood. However, the figures given and the conditions in which the insects were bred show that their results must be regarded as preliminary. To kill all these insects in 3 to 4 days, doses of 193,000 to 257,000 r are necessary. *Lasioderma* and *Sitophilus* which received 16,100 r did not die sooner than the controls, and 64,400 r is needed to kill all *Rhizopertha* in 20 days. It can therefore be assumed that the dose of x- or  $\gamma$ -rays needed to kill an insect is about 100 times greater than that needed to kill a mammal.

(iv) The other invertebrates are less well known. The larvae of *Trichinella spiralis*, an intestinal and muscular parasite of many mammals, are resistant to 3250 to 3750 r but they no longer thrive after ingestion by rats, remaining confined to the intestines<sup>11</sup>.

Embryos of the American squid (*Loligo pealeii*) irradiated before emerging from the ovum, do not succumb to a dose of 200,000 r of x-rays until the fourth day. Irradiation with 50,000 r causes swelling of the capsule of the ovum and liberation of all the embryos. The positive phototropism of many embryos is abolished by 10,000 r and growth is stopped by 5000 r, yet the muscles of the chromatophores continue to pulsate regularly even after 200,000 r. The most radiosensitive organ of the squid seems to be the crystalline lens<sup>12</sup>.

The Coelenterates, except the hydrae, are apparently resistant to cyanide (0.4 per cent); they are also radioresistant and about 300,000 r (40 kV) are needed to cause complete killing of the actinid *Anemonia sulcata*. The death rate after 100,000 r does not differ significantly from that of the controls<sup>13</sup>. On the other hand, the hydrae, which are much more sensitive to cyanides, are also much more sensitive to radiations<sup>14-15</sup>. If brown hydrae are irradiated (9600 r measured in water by chemical methods, 50 kV unfiltered radiations) there is severe degeneration of the tentacles after 24 hours in 28 out of 30 animals<sup>15</sup>. This is an indirect effect; apparently the

\* *Attagenus* (larvae and adults), *Lasioderma*, *Rhizopertha*, *Tribolium*, *Dermestes* (larvae and adults).

## COMPARATIVE RADIOSENSITIVITY OF LIVING ORGANISMS

irradiation of the water (or weak saline solution) *in the presence of oxygen* causes the formation of peroxides which are toxic for hydrae<sup>15</sup>.

Dr P. B. Pearson, Chief of the Biology Branch, Division of Biology and Medicine, U.S. Atomic Energy Commission, kindly communicated to us the interesting data known to him. They are collected in *Table II*. Further research with invertebrates is desirable, as it would be useful to understand their resistance to ionizing radiations.

The ova and spermatozoa of invertebrates have always been widely used in biological work. Those of a lamellibranch (*Spisula solidissima*)<sup>19</sup> and those of an urchin (*Arbacia*)<sup>20</sup> are very resistant to radiations, especially when concentrated, for it is usual to employ doses of 100,000 to 300,000 r when working with these animals. In 1940 EVANS and his collaborators<sup>21</sup> demonstrated the great influence of the medium on the results of irradiation of the spermatozoa of *Arbacia punctulata*. The greater the concentration of the sperm, the greater is its resistance to radiations\*. The seminal fluid in which the spermatozoa are suspended protects them from the rays. Heavily irradiated sea water diminishes the fertility of spermatozoa. This loss of fertility is certainly an indirect effect, as is the diminished rate of cell division in ova fertilized by irradiated spermatozoa and may be due to peroxides<sup>22</sup>.

(v) The higher plants are also very variable as regards radiosensitivity. Some authors relate this to their ascorbic acid content (see p. 72), and others believe that the fragility and length of the chromosomes are of fundamental importance.

The radiosensitivity of dry seeds varies from 2000 to 64,000 r (x-rays). Lily seeds (*Lilium regale*) show practically no growth† after 2000 r, whereas the seeds of cabbage and radish are practically unaffected by 64,000 r. In many cases a small dose (2000 to 8000 r) stimulates growth<sup>18</sup>. Many examples have already been given of the radiosensitivity of the pollen of certain plants (especially *Tradescantia*) (see p. 163), of the growing roots of the onion (*Allium cepa*), and of the broad bean, which are the best material for certain biological experiments.

The algae are certainly very resistant to radiations as BLINKS<sup>17</sup> found the whole algal flora abundant and in good anatomical and physiological condition on the most radioactive reefs at Bikini. The only abnormality found was a great increase in the catalase content of three genera (*Halimeda*, *Udotea* and *Cladophoropsis*). The question arises whether this is an enzymatic adaptation brought about by the formation of large quantities of peroxides when the bomb exploded.

---

\* This is also true of the sperm of *Nereis*, a marine annelid<sup>21</sup>.

† Seeds must be regarded as embryos.

## INTERPRETATION OF DIFFERENCES IN RADIOSENSITIVITY

### INTERPRETATION OF DIFFERENCES IN RADIOSENSITIVITY

In the present state of our knowledge these enormous differences in radiosensitivity certainly cannot be explained, but certain hypotheses deserve brief discussion.

(i) The relation between resistance to radiations and resistance to cyanides, demonstrated by BACQ, MUGARD and HERVE<sup>16</sup>, suggests that the enzyme systems sensitive to cyanide are also the most sensitive to radiations. If this fact were demonstrated in insects and other invertebrates, it would be an indication that the first enzyme systems to be affected are those containing heavy metals (Cu, Fe etc.).

(ii) The resistance of adult insects to radiations cannot be interpreted as a result of the fact that cell division (mitotic activity) no longer takes place in these animals except in the sexual cells. The sexual cells of insects are, in fact, as resistant in comparison with mammalian sexual cells as the whole body. For example, female rats protected by cysteamine are permanently sterilized by 700 r whereas 64,000 r are needed to produce the same result in *Drosophila*; in this insect 8000 r causes sterility only after 4 days, whereas temporary sterility in mammals is produced by doses of 150 to 200 r.

(iii) So far, no attempt has been made to correlate two facts which are now well established. (a) Most invertebrates maintain their intracellular osmotic pressure by means of amino-acids or small polypeptides\*<sup>29, 30</sup>. The internal environment of insects is very rich in amino-acids. Vertebrates, on the other hand, maintain their osmotic balance almost entirely by the interplay of inorganic ions such as:  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{SO}_4^{--}$ .

(b) As amino-acids have some protective action (see p. 301), it seems possible that at least a part of the resistance of invertebrates to radiations may be explained by this biochemical consideration, which might well form the basis of an experimental study†.

In insects the oxygen supply is brought by tracheae. It is possible that this anatomical arrangement results in a low oxygen tension in the tissues. Certain insects secrete large quantities of carboxylic acids (*e.g.* formic acid), which are good protectors against radiations.

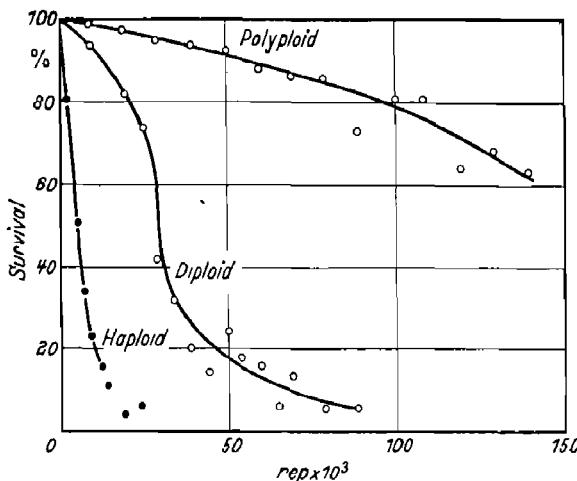
(iv) The number of chromosomes is very important. Among the yeasts, the diploid races are much less sensitive to u.v. and ionizing radiations of all kinds. Moreover, the mortality curve of diploid

\* Sometimes even by means of amines, such as taurine in the cephalopods.

† The fact that certain cancer cells are able to concentrate amino-acids<sup>31</sup> might affect their radiosensitivity. It would be interesting to know the radiation resistance of the octopus since it has a gland containing hydroxytryptamine which was found on a weight basis to be the most effective protective agent known (see p. 303).

### COMPARATIVE RADIOSensitivity OF LIVING ORGANISMS

yeasts is sigmoid in form, whereas it is straight in haploid yeasts. This fact has been very well observed by LATARJET and EPHRUSSI<sup>24,25</sup>, and it has been confirmed, extended to polyploid yeasts and studied in detail by TOBIAS<sup>26, 27</sup> (*Figure 1*). It is of great importance in general radiobiology. The advantage possessed by the polyploids is that they have several complete sets of chromosomes. In a haploid it is sufficient that one chromosomal region should be affected by irradiation to produce serious cellular changes. A similar lesion in a diploid is less important, because the corresponding region remains intact in the other series of chromosomes. This idea has been developed quantitatively<sup>25, 26</sup>. The sigmoid form



*Figure 1. Effect of x-rays on the survival of yeast cells containing different numbers of chromosomes<sup>26</sup>*

of the mortality curve of diploid yeasts suggests a two-hit phenomenon (see p. 59).

Several researches have established the fact that the liver of rodents is more or less polyploid. This is shown by the cytological studies of D'ANCONA and his collaborators<sup>32</sup> as well as by the histophotometric studies of PASTEELS and LISON<sup>28</sup>. SWIFT<sup>33</sup> has confirmed the existence of three types of nuclei in the liver, in which the DNA content increases by doubling. In adult male rats tetraploids are numerous, whereas there are hardly any in new-born animals. Unpublished observations by PASTEELS (1954) show a remarkable gigantism of the hepatic cells in castrated female rats; these are probably cells of very high polyploidy. It would be very interesting to extend these observations to other organs and to discover whether polyploidy in the liver is not partly responsible for the greater resistance of adults to radiation. The fact that the number of

## INTERPRETATION OF DIFFERENCES IN RADIOSENSITIVITY

chromosomes in certain tissues in vertebrates varies with the physiological conditions deserves the attention of radiobiologists.

As far as we know no experiments on plants have been conducted in spite of the very remarkable natural and induced polyploidy of certain solanaceous plants (tomato, potato), primulas and roses. In animals, tetraploids and other polyploids are much rarer; it can hardly be hoped to raise more radioresistant races by doubling or quadrupling the number of chromosomes in mammals.

### REFERENCES

- <sup>1</sup> BONET-MAURY, P. and PATTI, F., *J. Radiol. Electrol.*, 1953, **34**, 636
- <sup>2</sup> RAJEWSKY, B., HEUSE, O. and AURAND, K., *Z. Naturf.*, 1953, **8B**, 157
- <sup>3</sup> FORSSBERG, A., *Acta Radiol.*, 1941, **22**, 252
- <sup>4</sup> RUGH, R., *Milit. Surgeon*, 1953, **112**, 395
- <sup>5</sup> BRAND, TH. VON, *Biodynamica*, 1945, **5**, 353
- <sup>6</sup> RUGH, R., *J. exp. Zool.*, 1949, **110**, 357
- <sup>7</sup> RUGH, R., *Contr. AT-30-I-Gen.* 70, Oak Ridge, May 1949
- <sup>8</sup> PHYLLIS, S. S., *J. exp. Zool.*, 1950, **115**, 25
- <sup>9</sup> SULLIVAN, R. L. and GROSCH, D. S., *Nucleonics*, 1953, **11**, No. 3, 21
- <sup>10</sup> HASSETT, C. C. and JENKINS, D. W., *ibid.*, 1952, **10**, No. 12, 42
- <sup>11</sup> LEVIN, A. J. and EVANS, T. C., *J. Parasit.*, 1942, **28**, 477
- <sup>12</sup> RUGH, R., *Biol. Bull.*, 1950, **98**, 247
- <sup>13</sup> BACQ, Z. M. and HERVE, A., 1952, unpublished
- <sup>14</sup> BRIEN, P., 1953, personal communication
- <sup>15</sup> DANIEL, G. E., and PARK, H. D., *J. cell. comp. Physiol.*, 1951, **38**, 417
- <sup>16</sup> BACQ, Z. M., MUGARD, H. and HERVE, A., *Acta Radiol.*, 1952, **33**, 489
- <sup>17</sup> BLINKS, L. R., *J. cell. comp. Physiol.*, 1952, **39**, Suppl. 2, 11
- <sup>18</sup> SAX, K., *AAAS Symp. Radiobiol.; Nucleonics*, 1952, **8**, No. 4, 28
- <sup>19</sup> RUGH, R., *Biol. Bull.*, 1953, **104**, 197
- <sup>20</sup> HENSHAW, P. S., *Amer. J. Roentgenol.*, 1940, **43**, 899
- <sup>21</sup> EVANS, T. C., SLAUGHTER, J. C., LITTLE, E. P. and FAILLA, G., *Radiology*, 1942, **39**, 663
- <sup>22</sup> EVANS, T. C., *Biol. Bull.*, 1947, **92**, 99
- <sup>23</sup> *Univ. Rochester Atom. Proj., UR-38*, 1948, p. 13
- <sup>24</sup> LATARJET, R. and EPHRUSSI, B., *C.R. Acad. Sci., Paris*, 1949, **229**, 306
- <sup>25</sup> LATARJET, R., *Symp. Radiobiol.* (ed. J. J. Nickson), Wiley, New York, 1952, p. 241
- <sup>26</sup> TOBIAS, C. A., *ibid.*, p. 357
- <sup>27</sup> TOBIAS, C. A., *Acta Radiol.*, 1954, **41**, 105,
- <sup>28</sup> PASTEELS, J. and LISON, L., *C.R. Acad. Sci., Paris*, 1950, **230**, 780
- <sup>29</sup> CHRISTENSEN, H. N., *Annu. Rev. Biochem.*, 1953, **22**, 241
- <sup>30</sup> CAMIEN, M. N., SARLET, H., DUCHATEAU, G. and FLORKIN, M., *J. biol. Chem.*, 1951, **193**, 881
- <sup>31</sup> CHRISTENSEN, H. N. and HENDERSON, M. E., *Cancer Res.*, 1952, **12**, 229
- <sup>32</sup> D'ANCONA, U., MAGRINI, M. and D'ANCONA, S., *Boll. Soc. Ital. Biol. sfer.*, 1949, **25**, 667
- <sup>33</sup> SWIFT, H. H., *Physiol. Zool.*, 1950, **23**, 169

## PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

To obtain a clear idea of the relative importance of the many observations published, it must be remembered *inter alia* that if the effect of ionizing radiations themselves are to be studied, the only valid biochemical observations are those made within a few hours of irradiation. The reasons for this are: (i) The fundamental primary chemical changes always *precede* the appearance of anatomically visible lesions. No biologist should forget that the maintenance of normal structure is the *result*, not the cause of chemical activity of the cells. Every effort must therefore be made to discover what is abnormal in an irradiated organism before the anatomists are able to identify anything. (ii) When dead cells accumulate in the lymphoid tissues (thymus, lymph glands, spleen etc.), as they do as soon as 2 hours after irradiation, it is of little use to make accurate chemical determinations. The living and the dead cells are mixed, and nothing more can be learned from chemical analyses than can be seen except with the microscope. (iii) It is useless to carry out systematic determinations of any substance for days or weeks when it is characteristic of a type of cell which can be counted. For example, the blood glutathione after an irradiation follows closely the variations in the number of red cells<sup>21</sup>. All the glutathione is in the red corpuscles, and chemical determinations tell us no more than an erythrocyte count. What is interesting is to know whether there is a decrease in the reduced glutathione in the blood or tissues *immediately* after irradiation. There is no such decrease<sup>18</sup>. (iv) As several authors have already noticed (see especially ORD and STOCKEN<sup>1</sup>), the biochemical study of supposedly radioresistant tissues (especially the liver and kidney) is more valid because there is less interference by ordinary gross anatomical reactions. One can also follow the example of FORSSBERG and KLEIN<sup>127, 128</sup> and give a dose of ionizing radiations which do not kill any cells. (v) Biochemical investigation immediately after irradiation is advantageous in every way: the animals are still in good condition, suffering neither from shock, infection, nor from the severe malnutrition caused by vomiting, anorexia\* and diarrhoea. In the

---

\* Anorexia = loss of appetite.

## PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

final stages of radiation-sickness all observations are invalidated by these non-specific disturbances, because the body reacts against them, and these secondary reactions interfere with the primary reactions caused by the ionizing rays. Our attention is therefore concentrated on those researches in pathological biochemistry which show what happens immediately after irradiation. The reader's attention is directed to the review by ERRERA which summarizes the available data for immediate inhibition of enzymes *in vivo*<sup>142</sup>.

### OXYGEN CONSUMPTION

This is not affected in the guinea-pig by a whole-body dose of 250 r of x-rays (LD30)<sup>3</sup>. In starving rats, the basal metabolism is somewhat higher after irradiation than in fasting controls; there is no difference if the rats are not starving<sup>4</sup>. According to MOLE<sup>5</sup> there is no significant rise until 4 days after irradiation with 800 r, a lethal dose. The earlier observation of KIRSCHNER *et al.*<sup>6</sup> that the basal metabolism is increased by 30 to 60 per cent during the first 24 hours after a lethal irradiation, has not been confirmed. MOLE<sup>5</sup> observed no change in oxygen consumption in rats after a dose of 1000 r, nor is this changed in minced brain of mice either immediately or 190 hours after a whole-body irradiation with 500 r<sup>130</sup>. Respiration of dormant dry barley seeds (irradiated dry and germinating at 20°C immediately after irradiation) is not affected by 2500 r of x-rays; even doses of 5000 to 15,000 r stop after 4–5 days only the normal increase of O<sub>2</sub> consumption observed in controls<sup>131</sup> on germination.

Thus it seems that the principal enzymatic and hormonal systems which regulate metabolism and cellular respiration are not greatly affected by small or medium doses of x-rays; or if certain enzymatic processes are blocked by the irradiation alternative mechanisms immediately come into play.

Anatomical changes in the thyroid and considerable variations in its uptake of iodine, have been described after irradiation<sup>1</sup>, in particular there is an increase two hours after irradiation. The significance of this is not clear, as this is not accompanied by a general increase in metabolism<sup>122</sup>. It appears that irradiated animals which survive irradiation have a more active thyroid, whereas the opposite is observed in dying animals; castration prevents the thyroid hyperactivity<sup>123</sup>. Some workers claim that hypothyroidy increases mortality, anaemia and leucopenia<sup>124</sup>, but these facts have not been encountered by other authors<sup>123, 125</sup>. A detailed bibliography of morphological changes of the thyroid after irradiation is given by COMA<sup>126</sup>.

## PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

## CARBOHYDRATE METABOLISM AFTER IRRADIATION

This aspect of radiobiology was reviewed by Ord and Stocken in 1953, and we shall add only a few comments and analyse some of the most recent researches.

Enormous doses of x-rays (110,000 r, survival 3½ hours) clear the liver of its glycogen reserves<sup>8</sup>. On the other hand a moderate dose of x-rays (*c.* 300 r) in rats which were kept starving before and after irradiation brought about a considerable and steady increase in the glycogen content of the liver during the hours following irradiation<sup>9, 10, 11</sup>. This accumulation of glycogen in the liver is inhibited by the injection of cysteamine (mercaptoethylamine) or ingestion by mouth of cystamine (*Table I*).

*Table I. Variation in Liver Glycogen in Adult Starving Rats<sup>10</sup>*

<i>Group</i>	<i>Hours of fasting before irradiation or injection</i>	<i>Total duration of fasting: Hours</i>	<i>Killed after irradiation or injection: Hours</i>	<i>Number of rats</i>	<i>Liver glycogen</i>
<i>Controls</i>	—	32	—	10	2.8 (2.2 to 4.9)
	—	56	—	8	5.25 (2.9 to 8)
<i>Injected with 10 mg/100 g of cysteamine only</i>	24	32	8	6	2.1 (1.4 to 2.7)
	24	56	32	6	2.6 (2 to 3.5)
<i>Irradiated with 800 r only</i>	24	32	8	10	6.7 (4 to 11.2)
	24	56	32	10	22.3 (17 to 28.9)
<i>Injected with 10 mg/100 g of cysteamine before irradiation with 800 r</i>	24	32	8	8	2.6 (2.2 to 3.2)
	24	56	32	10	7.1 (5 to 9.1)

Since it is known that cortisone increases glycogen accumulation in the liver the interference of the pituitary-adrenal system, stimulated by stress, cannot be excluded<sup>12, 10</sup>. HERBERT and MOLE<sup>70</sup> observed that adrenalectomy prevents the rise in liver glycogen in the irradiated rat. During the first 2 hours after a dose of 2000 r in mice, the liver takes up three times as much <sup>14</sup>C in glycogen or a precursor as the controls after a subcutaneous injection of labelled glucose<sup>13</sup>. In mice irradiated with 2500 r there is also an increase of the incorporation of <sup>14</sup>C in the fraction of the hepatic lipids, the metabolism of which is very rapid. The decrease (20 per cent) of the peripheric use of glucose by the irradiated mouse<sup>7</sup> is not enough

#### CARBOHYDRATE METABOLISM AFTER IRRADIATION

to explain these facts. LOURAU<sup>13</sup> thinks that catabolism of the proteins, notably those derived from the intestine, might produce excess amino-acids which then become an important source of hepatic glycogen. In Fischer's experiments<sup>10</sup> the material used in the synthesis of glycogen certainly did not come from the food, since the animals were kept starving. It seems likely (but not proved) that the carbohydrate was synthesized from compounds having 2 or 3 carbon atoms produced by the catabolism of fats or proteins. The total nitrogen in the liver is not changed after irradiation. At first sight it seems improbable that the elements necessary for this synthesis of glycogen are obtained by increased break-down of liver proteins since fat tends to accumulate in the liver of rodents after irradiation. It is probable therefore that the metabolites incorporated in the liver glycogen during the hours following irradiation are brought to the liver by the blood.

LOURAU and LARTIGUE<sup>11</sup> found deficient glycogenesis in guinea-pigs 12 to 14 days after a non-fatal irradiation (500 r) and put forward the hypothesis that irradiation permits the synthesis of glycogen from alimentary carbohydrates but inhibits synthesis from smaller molecules, which differs considerably from those seen during the first 24 hours after irradiation. ORD and STOCKEN<sup>1</sup> confirm that the deposition of glycogen in the liver of the guinea-pig usually occurs 24 hours after irradiation, but not 6 to 8 days later.

There seems to be no great change in the absorption of glucose by the intestine, in spite of the bad condition of the intestinal mucosa shortly after irradiation\*. Glucose absorption only becomes slower during the 24 hours which follow irradiation<sup>37</sup>. In rabbits there is distinct hyperglycaemia reaching a maximum 2 to 4 hours after a dose of 500 r, the blood sugar returning to normal in 24 hours. After 1000 r the increase in plasma glucose reaches 90 per cent<sup>45</sup>. After 2000 r hyperglycaemia amounting to 3 to 5 g/litre is observed. This hyperglycaemia is easily blocked by insulin<sup>46</sup>. Early hyperglycaemia is also seen in guinea-pigs<sup>100</sup> and is more marked if the liver is irradiated directly<sup>37</sup>. In dogs increase in blood sugar produced by the ingestion of 1 g/kg of glucose is less pronounced for 3 days after irradiation with 450 r, which is probably due to slower intestinal absorption<sup>47, 37</sup>. The concentration of lactic acid and pyruvic acid in the blood is less than usual<sup>37</sup>. This last observation

---

\* In mice there are distinct lesions in the epithelium of the small intestine after less than an hour. Regeneration is evident by the third day and this is perhaps the tissue which regenerates most quickly. In fact, most mice which have received a fatal dose (700 to 800 r) die after the intestinal epithelium has already achieved a measure of regeneration.

### PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

is surprising, for if certain enzymatic systems were blocked in the carbohydrate cycle one or other of the metabolites should accumulate and an increase in bisulphite-binding substances\* has only been reported in the urine of irradiated cancer patients<sup>38</sup>. In mice, glycaemia is normal for the first hour following a whole body dose of 2000 r<sup>13</sup>.

DUBOIS, COCHRAN and DOULL<sup>39</sup> use fluoroacetic acid, according to POTTER's<sup>41</sup> method to determine whether citric acid is formed in irradiated rats in the same way as in normal rats. As has been shown by the investigations of SIR RUDOLPH PETERS, BUFFA and WAKELIN<sup>40</sup>, fluoroacetic acid is converted by the body into fluorocitric acid, which blocks Krebs cycle at the citric acid stage, and this substance accumulates. In rats injected with fluoroacetate 20 minutes after irradiation and killed 3 hours later, there is a great fall in the citric acid content of the thymus and spleen (radiosensitive organs) and also in the kidney† the decrease being greatest 6 to 12 hours after irradiation.

*Table II.* shows that the irradiated pancreas has also lost its ability to form citrate, but that the heart and brain (radioresistant organs) continue to accumulate the same quantity of citric acid. It is interesting to study the behaviour of the liver, in view of its importance in the reaction to radiation sickness. *Table II* shows that, unlike all other organs, the liver of male irradiated rats accumulates more citric acid than the controls after fluoroacetate poisoning.

*Table II. Effect of x-rays on the Accumulation of Citric Acid in the Tissues of Rats poisoned with Fluoroacetate (after DUBOIS, COCHRAN and DOULL<sup>39</sup>)*

Organ	μg citric acid per g fresh tissue	
	Controls	48 h after 800 r
Ileum . . . .	736 (680 to 816)	147 (94 to 176)
Pancreas . . . .	884 (704 to 1064)	76 (50 to 109)
Testicles . . . .	168 (150 to 225)	79 (61 to 110)
Skeletal muscle . . . .	88 (60 to 104)	58 (45 to 79)
Liver . . . .	64 (44 to 71)	177 (100 to 384)

Normally the liver of the adult male and young female rat seems to accumulate little citric acid, whereas that of the normal adult female accumulates large quantities (up to 1.5 mg/g) after an injection of

\* Pyruvic acid, acetaldehyde, methylglyoxal and generally all substances having an aldehyde or ketone group combine with bisulphite.

† The citric acid in the kidneys comes mainly from the blood: the kidneys concentrate for excretion the citric acid produced in the other tissues.

#### CARBOHYDRATE METABOLISM AFTER IRRADIATION

fluoroacetate. DUBOIS *et al.*<sup>39</sup> express surprise at the variability of the citric acid content of the liver of male rats poisoned with fluoroacetate. BEAULIEU and DALLEMAGNE<sup>42</sup> claim to have shown that the reason for this behaviour is that the liver destroys large quantities of citric acid after death. By taking the precaution of killing the rat, removing the liver, weighing it and crushing it in trichloracetic acid within 30 seconds, an average of 1.8 mg is found 2 hours after the injection and 2.0 mg 8 hours after the injection of fluoroacetate. It follows, firstly, that the liver of the male rat contains a system which anaerobically destroys the citric acid accumulated after fluoroacetate poisoning, and secondly, that this system according to the experiments of DUBOIS *et al.*<sup>38</sup> is partially inhibited by irradiation. This deserves further detailed study, especially as certain steroid hormones must be implicated.

Using <sup>14</sup>C labelled methanol HEVESY<sup>133</sup> has shown that neither the complete utilization of minute ( $0.3\mu$  g/g body weight) nor that of large doses ( $530\mu$  g/g) of methanol is depressed by exposure of mice to an x-ray dose of 1500 r immediately or one day before injecting the animals. In fact the metabolism of large doses of methanol is slightly increased<sup>133</sup>.

The ciliated protozoon *Tetrahymena pyriformis* after  $3 \times 10^5$  r of x-radiation oxidizes greatly increased amounts of acetate, but is unable to oxidize phenylalanine at the same rate as the controls<sup>135</sup>.

#### DISTURBANCES IN FAT METABOLISM

*Hydroperoxides*—Fats provide experimental opportunities in radiobiology which carbohydrates do not. *In vitro*, unsaturated fatty acids (e.g. linoleic acid) are very sensitive to radiations, being oxidized to form hydroperoxides. In the presence of oxygen the ionic yield in aq. solution is 103, whereas without oxygen it is only 9. Cysteine protects linoleate<sup>48</sup>. Hydroperoxides formed from lipids are more stable both *in vivo* and *in vitro* than hydrogen peroxide, and one might hope to find measurable quantities of organic peroxides immediately after irradiation in extracts of animals. The first difficulty is that the fatty tissues and even the natural lipids extracted from mammals are much less sensitive to x-rays\* than pure fatty acids *in vitro*, because of the presence of  $\alpha$ -tocopherol (vitamin E), which is a powerful antioxidant. In spite of this, the three groups of workers who have looked for hydroperoxides have found them, and their finding is one of the few chemical observations in

---

\* They are more easily affected by u.v. light.

radiobiology about which there is complete agreement (*i.e.* an oxidation which had been predicted from *in vitro* radiochemical studies). Thus, DUBOULZ *et al.*<sup>49</sup> state that fat in the skin of rats contains peroxides after intense local irradiation by u.v. light, or after 'contact' irradiation with soft x-rays, whereas normal skin contains none. Some of the figures (expressed as  $10^{-8}$  equivalents of oxygen) are: 2.25 immediately after intense u.v. irradiation; 0.58 immediately after 500 r x-rays; 1.16, 2 hours after 3250 r. These findings cannot be interpreted as a direct radiochemical oxidation brought about by free radicals, or by the direct action of x-rays as: (i) The peroxide level increases with time (*e.g.* goes up from 1.16 after 2 hours to 2.96 days after 3250 r); (ii) simple burns bring about the immediate appearance of more peroxide than is produced by 5000 r and there can be no doubt that these substances are lipid peroxides<sup>51</sup> and they readily oxidize the —SH of proteins<sup>53</sup>; (iii) a group of workers at Strasbourg<sup>54</sup> and another at Harwell<sup>55</sup>, using Hartman and Glavind's method, determined the lipid peroxides in the fat of rats<sup>54</sup> and in whole mice<sup>55</sup> immediately after total exposure to 1000 r, and found  $0.38 \times 10^{-3}$  milliequivalent/g of fat in the rat and  $2.27 \times 10^{-7}$  mole/g of mouse. This is a surprisingly high value and corresponds to an ionic yield of 84 if all the energy absorbed is used solely in this reaction. This evidence taken together leaves little doubt that this oxidation is not a primary effect of the ionizing radiation *in vivo* but an 'end-effect' resulting from biochemical changes.

If  $\alpha$ -tocopherol inhibits the formation of peroxides, and the lipid peroxides formed by ionizing radiations are the cause of cellular lesions, one would expect that injection of  $\alpha$ -tocopherol or a diet rich in vitamin E would increase resistance to radiation. This is not the case<sup>54, 129</sup> because, as we believe, a normal diet ensures satisfactory concentration of tocopherol in fat. It would be more instructive to look for a progressive decrease in resistance to radiations in the course of tocopherol deficiency, but this has not been tried, although preliminary observations by Bacq and Herve suggest that tocopherol deficiency increases the radiosensitivity of mice.

Peroxides<sup>57</sup> show many 'radiomimetic' properties (see p. 191) but many enzymes (*e.g.* carboxypeptidase) are not inhibited nor can some of the cellular effect of x-rays be reproduced by hydrogen peroxide. ALPER<sup>58</sup> rightly draws attention to an error introduced by the fact that most commercial hydrogen peroxide is contaminated with different stabilizers. These are not easily removed by distillation, and considerably alter the biological effects of  $H_2O_2$ . For instance, the effect on a phage of a very pure preparation of  $H_2O_2$  (obtained by 15,000 r of x-rays) in one part per million was repro-

#### DISTURBANCES IN FAT METABOLISM

duced only by a concentration 50 times greater of a redistilled commercial peroxide.

Some authors<sup>59</sup> consider that peroxides play a part in carcinogenesis. One wonders whether ascorbic acid plays the same anti-oxidizing part in aqueous systems as  $\alpha$ -tocopherol plays in fat. In animals the excretion of dehydroascorbic and diketogulonic acids is increased after irradiation (see Chapter 12). In plants exposed to continuous non-lethal irradiation (5000 r per day) the ascorbic acid content falls for the first two days, and then increases again. There may be some relation between a high ascorbic acid content and radioresistance in green plants. The cabbage and *Gladiolus*, which are highly resistant, contain 200 to 400 mg of ascorbic acid per 100 g of fresh plant, whereas cosmos, tobacco and henbane\*, which have little resistance, contain only 30 to 100 mg of ascorbic acid<sup>52</sup>. On the other hand *Vicia faba* is very radiosensitive (85 per cent growth inhibition by 140 r) and is rich in ascorbic acid. In animals the reverse appears to apply; the organs (ovaries, adrenals) and tumours richest in ascorbic acid are also the most radiosensitive. Attempts to protect animals and human beings against the effect of x-rays or to treat radiation sickness by means of ascorbic acid, alone or together with cysteine, have sometimes been strikingly successful<sup>63</sup>, but generally the results have been very variable<sup>64</sup>. These experiments should be repeated in animals such as guinea-pigs, some strains of which are unable to synthesize ascorbic acid.

*Absorption and transport of lipids*—It is certain that there are disturbances in fat metabolism and transport in irradiated animals. In rabbits, a large dose (1000 to 2000 r) of x-rays causes very considerable lipaemia (+600 per cent) in 24 to 48 hours, which disappears on the third day. The plasma is opalescent and creamy and its total lipid, phospholipid and cholesterol content is very high<sup>60, 61, 62</sup>. This phenomenon is peculiar to rabbits, and is rarely observed in rats and dogs; in guinea-pigs it is regularly found, but is less than in rabbits<sup>61</sup>. In mice, Low-BEER<sup>65</sup> has always found a fall in blood cholesterol in the fourth hour after irradiation (300 to 1000 r); in guinea-pigs the cholesterol level rises 4 to 5 days after 200 to 600 r<sup>72</sup>. The blood cells also become rich in fat, but the increase is not parallel with that in the plasma<sup>61</sup>. These observations in rabbits, together with the hyperglycaemic acidosis, observed, make up a picture reminiscent of severe diabetes, and *Table II* also suggests that the pancreas is injured. Insulin, which reduces the hyperglycaemia produced by irradiation, also reduces the lipaemia produced

---

\* *Cosmos sulfureus*, *Nicotiana rustica*, *Hyoscyamus niger*.

PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

by 1000 r, but after a dose of 2000 r insulin no longer affects the lipaemia<sup>61</sup>.

The absorption of fats and fat-soluble vitamins (especially vitamin A) is reduced during the 24 hours which follow irradiation, and then becomes normal. There are general disturbances, but they vary from one species to another, and no general trend is evident. BACQ, BURG, CHEVALLIER and HEUSGHEM<sup>54</sup> have carried out a prolonged study in rats, which they nevertheless consider to be preliminary, in that it determines the exact problems which must be solved. The animals must be fasting in order to avoid the great variations, due to the very variable *anorexia* which follows irradiation. The reduction in total lipids and in  $\alpha$ -tocopherol content, as well as the amount

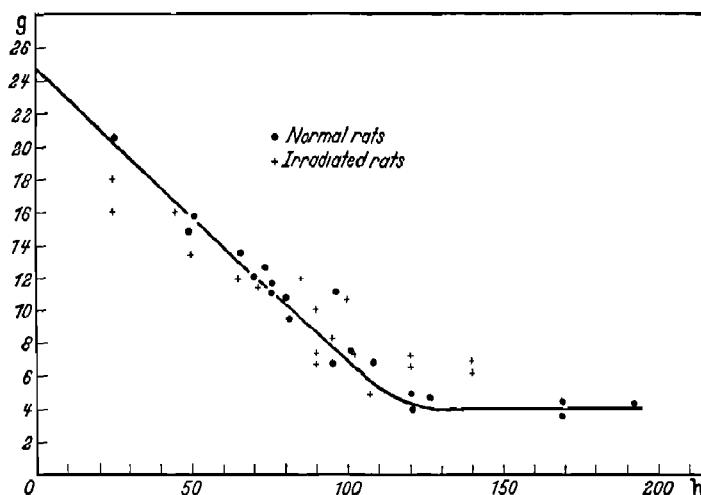


Figure 1. Changes in total fat content of starving rats produced by 1000 r of x-rays<sup>54</sup>

of unsaturation were compared in controls and irradiated animals (1000 r) in the whole body (excluding the head, tail and gut). The  $\alpha$ -tocopherol remains unchanged, in other words it is not destroyed by irradiation and is not utilized to a greater extent. It becomes gradually concentrated, as the fat reserves are mobilized. The total quantity of fats (22 g in a 200 g rat) falls rapidly during the first 70 hours after irradiation, both in the irradiated animals and in the controls. From the 70th to the 120th hour the total fat continues to fall in the controls, down to 4 g, but it remains at about 9 g in the irradiated rats. This means that after the 70th hour, either the synthesis of fat is increased in irradiated animals, or the utilization of fat is decreased.

#### DISTURBANCES IN FAT METABOLISM

The proportion of unsaturated fatty acids with 2, 3 or 4 double bonds<sup>54</sup> does not vary either in the controls or in the irradiated animals. These acids are regarded as having vitamin-properties, and play an essential part in metabolism; arachidonic acid, in particular, is an important constituent of the erythrocyte membrane.

*Liver fats*—These have often been studied and as early as 1945, ELLINGER<sup>36</sup> drew attention to the increased fat in the liver after irradiation. As this increase was lessened by deoxycorticosterone, he interpreted it as a sign of adrenal insufficiency. Recent work suggests the contrary explanation, as (i) an excess of cortisone constantly causes a deposition of glycogen and fat in the liver, and (ii) adrenalectomy in rats is not followed by an increase in fat in the liver, and prevents the fatty infiltration seen after irradiation<sup>66</sup>. It seems therefore that the accumulation of fats in the liver is the result of pituitary-adrenal overactivity, with an excess of gluco-corticoids in the blood as compared with mineralo-corticoids (see Chapter 12).

In starving rats irradiated with 1000 r there is no fatty infiltration (steatosis) of the liver. The weight of the liver and its total lipid content run parallel with those of starving controls. A great increase in liver fat is observed, irregularly, only if the irradiated animals are given a diet rich in fat<sup>54</sup>. The only way to produce true steatosis of the liver constantly in rats is to administer a dose of 50 r a day, which does not produce anorexia. The animals remain in excellent condition until shortly before death, when they have received a total dose of 1700 to 2350 r. At this stage the liver weighs less than in the controls, but its glyceride content is very high; 0.1 to 0.2 g/g fresh tissue<sup>67</sup>. It is too early to say whether this steatosis is due to a progressive lesion caused by irradiation or to cortical adrenal overactivity. The same clear picture does not emerge if one applies Chevallier's technique to mice.

There are several signs of increased phospholipid synthesis in the liver whereas the incorporation of <sup>32</sup>P in the phospholipids of the thymus remains unchanged after irradiation<sup>35</sup>, but caution is necessary in interpreting these experiments.

The increase in lipid synthesis from labelled acetate by the bone-marrow *in vitro* is discussed on p. 248, and the whole subject is reviewed by CHEVALIER and BURG<sup>143</sup>.

#### PROTEIN METABOLISM

Studies on protein metabolism abound in contradictions, even if late or terminal phenomena are disregarded. Some workers (*cf.* 1, 14) claim that there is great protein destruction in rats shortly after

irradiation (400 to 1000 r), which shows itself by an increase in the urinary excretion of total and urea nitrogen, but the blood urea of irradiated nephrectomized rats does not increase more than that of nephrectomized controls. The nitrogen balance is negative in young rats for two or three days after a non-fatal dose of x-rays (400 r), the absorption and retention of nitrogen being affected. LAMERTON and his colleagues emphasize that nitrogen excretion (urea, amino-acids and ammonia) is not affected qualitatively or quantitatively by irradiation in spite of the very distinct inhibition of growth<sup>79</sup>.

- Newly-hatched chicks occupy a special position in that they die after 1000 r with uric acid retention, caused not by increased protein catabolism but by renal failure<sup>69</sup>. Chicks which do not die soon after irradiation often die when 5 to 6 months old with ascites and cirrhosis of the liver<sup>75</sup>. Disturbances in transamination have been described, for example in the glutamic-aspartic acid system, the activity being reduced to 5 per cent of the normal, but this happens 4 to 6 days after irradiation, when serious anatomical changes have occurred. Pyridoxine, the co-enzyme of transamination, is not affected *in vivo*.

The cessation of growth after irradiation has led many authors to assume, without discussion or exact experimental evidence, that the synthesis of all proteins is slower after irradiation. This is an oversimplification. It will be shown (p. 248) firstly that the synthesis of haemoglobin is greatly accelerated before being slowed, and secondly that the changes in the synthesis of globin do not run parallel to those of haemin. Though it is asserted that deoxyribonucleic acid synthesis is strongly inhibited, the renewal of a histone and two protein fractions of a Jensen rat sarcoma is not affected consistently by 2000 r applied locally or to the body as a whole, judging by the incorporation of methionine labelled with one S<sup>35</sup> atom.

The ability of mammals to form antibodies is diminished by small doses (175 r) of x-rays (see in particular<sup>102</sup>), and this explains why infections are more dangerous in irradiated animals than in normal animals (see p. 284). The disturbances in the synthesis of enzymatic proteins are discussed on p. 245.

The concentration of the various plasma proteins of the rat does not change before the second day after irradiation, and the change is most marked on the third day after a dose of 600 to 1000 r. The fractions of plasma obtained by precipitation with cold ethanol have been carefully followed by analysis for total nitrogen, for fat, for cholesterol and electrophoretically. This excellent work<sup>71</sup> has shown

## PROTEIN METABOLISM

that the nitrogen content is increased in fraction IV - 4, which is rich in  $\alpha$ I-globulin and decreased in fraction V, which is rich in albumin. The lipids and cholesterol are also increased in fraction IV - 4. Electrophoresis showed that the quantity of  $\gamma$ -globulin of fractions II + III is always diminished. In dogs a diminution in albumin and globulin, coupled with an increase in total globulins is observed<sup>37</sup>. The change in the albumin/globulin ratio is the earliest pathological sign in the blood of rats after irradiation with 100 to 650 r, the increase being perceptible as early as 2½ hours after irradiation. Hyperglycaemia begins at the sixth hour, and hyperchloraemia and hypercholesterolaemia do not appear until the twelfth hour; the changes in the plasma proteins occur even in the absence of the pituitary or adrenals<sup>104</sup>. The interpretation of the changes is complicated by the presence of lipids<sup>99</sup>, by changes in the plasma volume<sup>103</sup>, and by differences between species and also between different strains of the same species<sup>104</sup>. If the changes in total plasma volume are taken into account, the total amount of plasma proteins in rats is seen to be diminished during the first week following total irradiation with 600 to 750 r; from the second week onwards the total amount is increased by 15 to 20 per cent above that in the controls, although the protein concentration of the plasma is less than normal; the total mass of plasma increases greatly (+ 40 per cent) after the seventh day<sup>103</sup>.

## CHANGES IN ELECTROLYTE CONCENTRATION

Besides the changes in K and Na ion balance mentioned in Chapter 12, severe hypocalcaemia (3 mg per cent) is found in rabbits, causing tetany and death 1 to 3 days after a dose of 2000 r. A curious and quite unexpected feature of this hypocalcaemic tetany is that it occurs during severe acidosis, whereas the hypocalcaemias observed in human and veterinary medicine (when there is no question of the action of ionizing radiation) is always associated with alkalosis. The acidosis of heavily irradiated rabbits appears in the fourth hour after irradiation<sup>62</sup>.

Although rabbits show peculiar reaction to x-rays, these observations deserve further investigation since a discussion of the detailed results obtained by KOHN<sup>102, 104</sup> in various strains of rats does not lead to any general conclusions. Some response of the rat such as the increase in blood cholesterol can be attributed partly to the adrenals, and some such as the increased chloraemia in immature rats, to the pituitary, but other reactions occur in the absence of these glands<sup>105</sup>.

## SULPHYDRYL ENZYMES AND PROTEINS

Sulphydryl groups occupy a special position in radiobichemistry for the following reasons: (i) The *in vitro* experiments of BARRON *et al.*<sup>14-16</sup> show rapid oxidation of the —SH groups of cysteine and BAL (=dimercaptopropanol), inactivation of the —SH enzymes, with more or less complete reactivation after the addition of cysteine or glutathione, *i.e.* physiological carriers of —SH (*cf.* p. 140). (ii) Many sulphydryl derivatives (cysteine, cysteamine) protect against radiations. This is in apparent agreement with the theory put forward as early as 1946 by Barron, that the fundamental lesion produced by ionizing rays *in vivo* is oxidation of the —SH of the intracellular enzymes and their inactivation, a theory based on the extension from *in vitro* to *in vivo* results. BARRON<sup>14</sup> and BARRON and SEKI<sup>17</sup> provide indirect evidence in support of this theory, *e.g.* the oxygen consumption of the ova and spermatozoa of a sea-urchin (*Arbacia punctulata*) is increased by small doses of x-rays (100 to 260 r) and inhibited by large doses. Similarly, a typical —SH blocking agent, mercuric chloride, increases the consumption of oxygen in small doses ( $5 \times 10^{-5}$ M), and inhibits it in large doses ( $1 \times 10^{-4}$ M). Both x-rays and mercuric chloride arrest mitosis in doses which increase oxygen consumption. The investigations of Hammett, Rapkine and Brachet have shown how important sulphydryl groups are in mitosis and embryonic development. Consequently Barron's theory received wide attention<sup>22</sup>.

However, against these indirect arguments the number of negative results from *in vivo* experiments which have appeared in recent years is so great that it seems impossible to accept Barron's theory as a whole<sup>122\*</sup>.

(i) If x-rays oxidize —SH either to S—S or further to SO<sub>2</sub>, one might expect† an immediate fall in reduced glutathione, and in total —SH groups in the blood and tissues after irradiation. Our collaborators showed in 1950 that there is no fall in reduced glutathione

\* This conclusion does not in any way diminish the value of Barron's experiments *in vitro*. L. MASSART<sup>19</sup>, has confirmed that x-rays inactivate papaine (an —SH enzyme not studied by Barron), that 0·01 per cent cysteamine protects papaine completely, and that cysteamine, when added to partially inactivated papaine, fully restores its activity.

† If every free radical produced by 700 r (a lethal dose) of whole-body irradiation of a mouse were used only to oxidize —SH groups (*e.g.* cysteine) the quantity of cysteine oxidized would be of the order of 25 µg; a change of this magnitude is within the experimental error of —SH determination. BRUES and PATT<sup>136</sup> also point out that the total amount of available —SH groups in the tissues is much greater than the quantity which might be oxidized during irradiation by a lethal dose.

## SULPHYDRYL ENZYMES AND PROTEINS

in the blood, liver, kidneys, heart and muscles of rats and guinea-pigs after 500, 600 or 700 r<sup>18</sup>. This has been fully confirmed<sup>24, 25, 26</sup>. The only observations showing a decrease in sulphhydryl groups were made long after irradiation, and the decrease does not precede, but follows the development of anatomical lesions<sup>18, 20, 21, 23, 24</sup>.

(ii) Not all the good protectors against radiation contain sulphhydryl groups, and many —SH compounds do not protect against x-rays; cystamine which contains an S—S bridge is an excellent protector (for details and discussion see Chapter 14).

(iii) Protectors against ionizing radiations which contain —SH groups are ineffective if injected after irradiation. This has been well shown by Patt *et al.* with cysteine, and by the authors with cysteamine. The time interval between irradiation and injection of the —SH compounds was kept extremely short; the lethal dose of x-rays was given to mice in 5 seconds, and the injection of cysteamine given within the next 20 seconds. The death rate in this experiment was the same as after irradiation alone, whereas the same dose of cysteamine injected immediately before injection gives a 100 per cent survival rate.

The fact that no great decrease in the total —SH groups is found by chemical titration does not mean that a certain —SH group carried in small quantities by molecules of great biological importance is not blocked. To take a precise example, supposing that CoA (in which the SH is carried by cysteamine) (see *Figure 2*) is completely oxidized in the tissues, this oxidation will not be noticed if the total —SH only is determined, because the sulphhydryl groups of this enzyme form only a minute proportion of the total —SH. Now the oxidation of CoA results in its inactivation, and the transfer of acetyl, succinyl, stearyl, and benzyl radicals is impossible, in other words there are disturbances of various kinds in the metabolism of carbohydrates (blocking of Krebs cycle), fat and perhaps protein, and these disturbances affect catabolism as well as many syntheses. It is naturally tempting to think of inactivation of CoA by ionizing radiations. The many changes seen after irradiation could then be explained by assuming only one biochemical lesion. Unfortunately there is no evidence to support this hypothesis.

(iv) It is impossible to demonstrate the inactivation of an —SH enzyme or coenzyme immediately after irradiation *in vivo* in the whole animal. For example, the acetylation of *p*-aminobenzoic acid in rats<sup>28</sup> or of sulphathiazol in rats and rabbits<sup>29</sup> is not blocked or even diminished if these substances are injected, during the hours which follow irradiation. It may be concluded that coenzyme A is not inactivated. Similarly, the succinodehydrogenase activity of

PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

homogenates of rat kidneys is unchanged 2 hours after irradiation with 400 or 800 r<sup>30</sup>. This fact has been confirmed in mice, not only in the kidney but also in the liver<sup>31</sup>. Homogenates of rat thymus retain all their dehydrogenase activity for succinic and malic acid, and their cytochrome oxydase and ATPase activity after irradiation with 800 r, although the decrease in concentration and the rate of incorporation of <sup>32</sup>P in desoxyribonucleic acid is very marked as early as 3 hours after irradiation<sup>35</sup>. Different results have been published for the spleen, another highly radiosensitive organ. ASHWELL and HICKMAN<sup>32</sup> and MAXWELL and ASHWELL<sup>33</sup> state that in rats the succinoxidase activity of the spleen is not

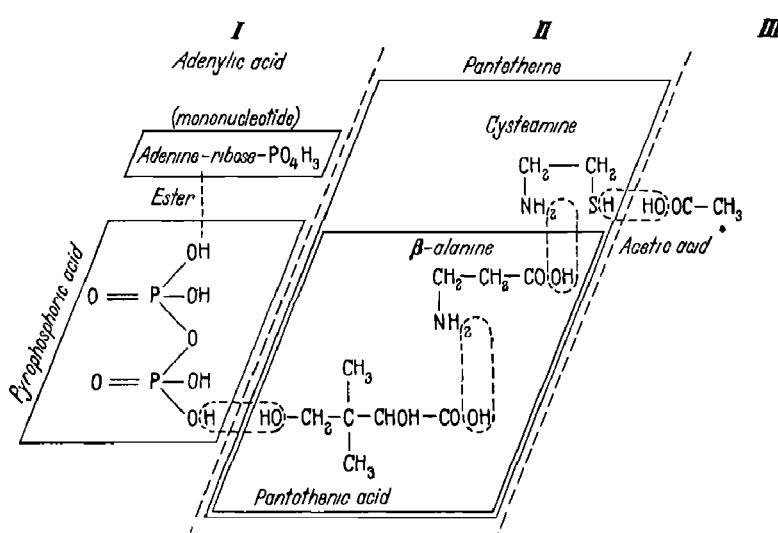


Figure 2 Structure of co-enzyme A

lowered between 4 hours and 17 days after irradiation; that is, whatever its state of degeneration or regeneration. In mice, the succinoxidase activity of the spleen seems to be diminished after irradiation, and this inhibition is prevented by cysteine even if it is injected after irradiation<sup>31</sup>.

DUBOIS *et al.*<sup>39</sup> stress the fact in their experiments the lethal dose of x-rays produced no appreciable decrease *in vivo* in the activity of succinodehydrogenase, adenosinetriphosphatase or other —SH enzymes in various tissues.

The most interesting fact which emerges from studies on homogenates of spleen is the great decrease in the ability of the splenic

## SULPHYDRYL ENZYMES AND PROTEINS

mitochondria to synthesize (or replace) the phosphorus compounds\* (energy rich) which are associated with the oxidation of succinate<sup>32, 37</sup>. Similarly, homogenates of the thymus of rats irradiated with 800 r have lost a great part of their ability to esterify inorganic phosphate<sup>35</sup>. In homogenates of the spleen of mice anaerobic glycolysis is reduced by 80 to 90 per cent after a dose of 650 r to the whole body, which is apparently due to the destruction of a phosphate acceptor<sup>43</sup>. If these facts are confirmed in other tissues, this may indicate a biochemical lesion, as ORD and STOCKEN<sup>1</sup> believe, in spite of their failure to confirm this fact in the *liver* of guinea-pigs. In this organ the ATP and phosphocreatine content remains normal for 6 to 8 days after irradiation; the adenylic acid, adenosinediphosphate and adenosinetriphosphate content of the blood of mice is also unchanged 2 to 8 days after a 100 per cent lethal dose of 800 r<sup>111</sup>.

Recent studies<sup>140, 141</sup> with homogenates (*i.e.* mitochondria) from rats or mice have confirmed: (i) ATPase activity is increased and oxidative phosphorylation decreased by a whole body dose of as little as 25 to 100 r if the homogenate is prepared within one hour following the irradiation. (ii) Neither of these changes is observed if the homogenate is irradiated *in vitro*. (iii) These changes are not observed in homogenates of the thymus or liver if these organs only are irradiated. In spite of the many detailed investigations which have been carried out the mechanism by which coupled phosphorylation is interfered with has not been found.

---

\* BRACHET<sup>116</sup> states: 'It is mainly, if not exclusively, in the *mitochondria* that *cellular oxidation* takes place, which provides the cells with the energy they need to carry out syntheses or work. But we know that the energy set free by cellular oxidation cannot be used directly by the cells, it must be accumulated in the form of the phosphorus compounds of adenosinetriphosphoric acid (ATP) which are rich in energy. It is the hydrolysis of these compounds, with the concomitant loss of one or two terminal molecules of phosphoric acid, which finally liberates the energy available to the cells. To make cellular oxidation really effective it must therefore be *coupled* with phosphorylation, that is, with endoenergetic fixation of inorganic phosphorus on a suitable acceptor (*e.g.* adenylic acid).

'Isolated mitochondria are capable of fulfilling these functions on condition that they are supplied with the materials which they have lost in the process of isolation. If, therefore, well washed mitochondria are prepared, and an oxidizable substrate (*e.g.*  $\alpha$ -ketoglutaric acid) is added, together with a co-dehydrogenase, an intermediate carrier of hydrogen (cytochrome c)  $Mg^{++}$  ions, inorganic phosphate, and a phosphate acceptor (adenylic acid), oxidation coupled with phosphorylation will be produced *in vitro*. The ketoglutaric acid will be oxidized, and inorganic phosphate will, at the same time, be fixed to the adenylic acid to form ATP. Thus the mitochondria function not only as catalysts for oxidation, but also continually replenish the stock of ATP, thus storing the energy set free by the oxidation in a form directly available to the cell. Finally, the mitochondria can also take part in the syntheses which find in ATP the energy needed for their production.'

## PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

### TRACER EXPERIMENTS WITH IRRADIATED ANIMALS

Hevesy and his collaborators have studied extensively the pathway of acetate labelled with a  $^{14}\text{C}$  atom in the carboxyl group, or of phosphate labelled with  $^{32}\text{P}$  after injection into animals<sup>2, 44</sup>. Hevesy makes no secret of the many difficulties which arise in interpreting the results. ‘The mapping out of a metabolic route by the use of  $^{14}\text{C}$  as an indicator is an arduous task. The task will be even more arduous if the effect of irradiation on this route is to be elucidated’<sup>2</sup>. One might almost say that the use of isotopes in biological research has raised more problems than it has solved. The  $^{14}\text{C}$  of acetate and the  $^{32}\text{P}$  in a phosphate go everywhere and serve many purposes; they are not a specific indicator like orotic acid in RNA synthesis. HEVESY<sup>2, 44, 112</sup> showed that the presence of an increased quantity of the isotope in a single fraction of an organ cannot always be interpreted as a reliable indication of renewal, or a more rapid ‘turnover’ in this fraction. He introduced the concept of the ‘sensitivity’ of an isotope indicator. The following quotation from HEVESY<sup>2</sup> illustrates its meaning.

‘Consider, for example, the formation of labelled liver lecithin following intravenous injection of  $^{32}\text{P}$ . In the first day as much lecithin will be turned over as in the second one, but because of the decrease in  $^{32}\text{P}$  in the inorganic phosphate pool with time the incorporation of the same amount of phosphorus into liver lecithin will be followed in the second day by the incorporation of appreciably less  $^{32}\text{P}$  than in the first day. One week before the administration of  $^{32}\text{P}$  one leg of a mouse was irradiated with roentgen rays, and the irradiated leg was found to take up only 70 per cent of the amount of  $^{32}\text{P}$  incorporated into the non-irradiated leg<sup>86</sup>. Thus the amount of  $^{32}\text{P}$  removed from the inorganic phosphate pool was reduced by altering the metabolic rate, in this instance by exposure to ionizing radiation. Correspondingly the liver lecithin of an irradiated mouse will incorporate more  $^{32}\text{P}$  in the course of a given time than does liver lecithin in a control mouse. The increased specific activity of the lecithin P in the irradiated animal will not be due, as one may be inclined to interpret, to an increased lecithin turnover in the liver of the irradiated animal. It is due to an interference of irradiation with the turnover rate of the mineral constituents of the skeleton, which makes  $^{32}\text{P}$  a less sensitive indicator of phosphorus in the irradiated mouse than in the control.’

A precise term should be adopted for this kind of radiation effect. It is neither an indirect effect (= free radicals) nor an effect at a distance (after irradiation); it is a *secondary* effect, a consequence of a primary biochemical lesion.

#### TRACER EXPERIMENTS WITH IRRADIATED ANIMALS

The same argument should be applied to acetate and bicarbonate ions and to glycine labelled with  $^{14}\text{C}$ . In spite of these limitations, the fact remains that the injection of isotopes in irradiated animals is a method which has revealed a number of primary, as well as distant and secondary effects of ionizing radiations<sup>2, 114</sup>. A few examples are given here, many will be found in Hevesy's work, in reviews especially<sup>1</sup> and in reports of recent symposia.

The injection of  $^{14}\text{C}$  glycine 48 hours after irradiation in rats gives rise to greater elimination of  $^{14}\text{C O}_2$  than in controls<sup>92</sup>. If one of two transplanted sarcoma in a rat is irradiated while the other is protected by a lead screen, the uptake of  $^{32}\text{P}$  is found to be reduced, not only in the irradiated tumour, but also in the shielded tumour<sup>115</sup>.

The significant data obtained, from the injection of acetate labelled with  $^{14}\text{C}$  in the carboxyl groups in fully *fed* mice are that an irradiation with 800 r *increases* the fixation of carbon; the increases are: +34 per cent in the dry brain tissue; +16 per cent in the brain proteins; +31 per cent in the brain fat; +12 per cent in the dry plasma; +12 per cent in the liver proteins.

These observations support our view that ionizing radiations accelerate metabolic processes (see p. 257). Forssberg and Hevesy, see <sup>2</sup>, repeated these experiments in *starving* mice, and did not observe these increases, the only significant finding being a decrease (-12 per cent) in the uptake of  $^{14}\text{C}$  by the liver lipids.

If glucose in which all the carbon atoms are labelled with  $^{14}\text{C}$  is injected subcutaneously in mice, it is found that the  $^{14}\text{C O}_2$  content of the exhaled air immediately after irradiation with 2000 r is 20 per cent less than in controls. Glucose catabolism is therefore diminished immediately after irradiation<sup>7</sup>.

#### INCREASED ENZYMIC AND SYNTHETIC ACTIVITY AFTER IRRADIATION

A number of reports from various radiobiological laboratories indicate increases in activity after irradiation *in vivo*, often of considerable magnitude (400 per cent) especially as the increase occurs very soon after irradiation. So far, painstaking searches have been carried out for inhibitions of enzymes, cessation of growth, and the slowing down of synthesis. The unexpected discovery of the astonishing early activation of radiosensitive tissues is, in our opinion, important, as it appears to be an essential phase in the course of events from irradiation to the death of the cells. No comparable increase has ever been observed after irradiation of enzymes *in vitro*.

## PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

These researches must be studied in detail in order to derive the greatest possible information. Many radiotherapists admit by implication that small doses of x-rays have a stimulating effect, for they use them to this end; but some radiobiologists have denied that there is any evidence to support this idea<sup>96</sup>. As early as 1924 ANCEL and VINTERBERGER reported that x-rays accelerate embryonic development<sup>97</sup> and Koller (see p. 163) has reported that the mitotic rate of *Tradescantia* is increased by low doses.

The experiments described below summarize the relevant data available in the literature.

(i) The increased activity of the tryptophane peroxidase-oxydase

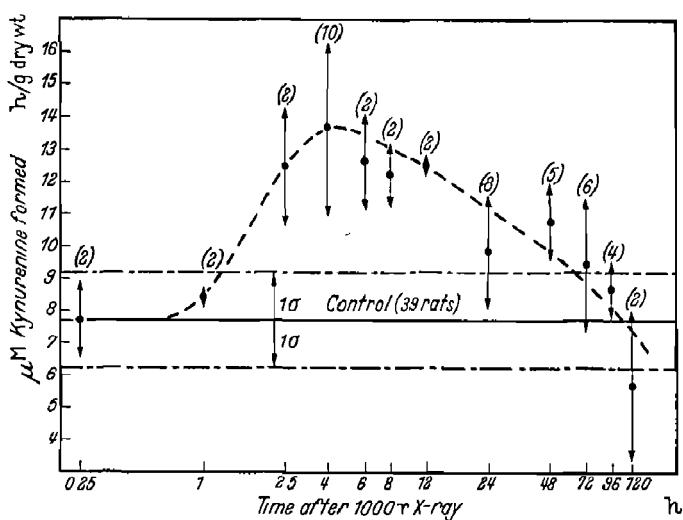


Figure 3. Effect of total-body x-radiation on rat liver tryptophane peroxidase-oxydase. The number of rats used at each point is given in parentheses. Vertical arrows represent the standard deviations<sup>74</sup>

system in the liver of rats after irradiation with 1000 r (Figure 3) depends on an adrenocortical reaction. This is an unusual enzymic system; it is an adaptive system as its concentration (or activity) increases rapidly if the animal is provided with an excess of tryptophane<sup>73</sup>. Adrenalectomy suppresses this reaction to irradiation, but hypophysectomy does not. The increased activity is not a response to stress in the sense of the adaptation syndrome but an action of the suprarenal cortex independent of the pituitary<sup>74</sup>.

(ii) In all mammalian tissues and organs there are not only several cathepsins (or carboxypeptidases), but also an inhibitor of these cathepsins<sup>76</sup>. Total irradiation of mice, rats and rabbits with

#### INCREASED ENZYMIC AND SYNTHETIC ACTIVITY AFTER IRRADIATION

about the 50 per cent lethal dose increases the activity of some of these cathepsins. In the case of the carboxypeptidase of the rat kidney, this increase is due to *destruction of the inhibitor by x-rays*. It is possible that certain blood cells (leucocytes) are the only source of this inhibitor, a determination of which, during the first 24 hours after irradiation would have some prognostic value<sup>77</sup>. These observations recall the fact reported by ABBERHALDEN in 1940<sup>78</sup> that proteases specific for the organs irradiated are found in the urine 6 to 9 hours after irradiation. They show that Euler and Hahn were right to emphasize that x-rays may have activating and inactivating effects on the same tissue homogenate, which may be considered to a half-way stage between the living cell and the pure enzyme. The mechanisms by which these contrary effects are brought about are probably quite different.

(iii) (a) The oxygen consumption of the ova and spermatozoa of *Arbacia punctulata* increases by 14 to 27 per cent in 2 hours after a small dose of x-rays (100 to 260 r)<sup>17</sup>. In baker's yeast, oxygen consumption and carbon dioxide anaerobic production is increased after a dose of 90,000 r although mitosis is inhibited by 20,000 r<sup>80</sup>. It is interesting that this dose of 90,000 r does not alter the uptake of glucose by these cells which no longer form colonies.

*Isolated systems after total irradiation of mammals*—Two complementary investigations provide much information. A research group at Rochester University (RICHMOND, ALTMAN and SALOMON), using homogenates of rabbit bone-marrow or spleen removed at various times after irradiation, have studied oxygen consumption and the incorporation of labelled atoms by various molecules or series of molecules<sup>87, 88, 89</sup>. The oxygen consumption, and the synthesis of haemin from glycine labelled with <sup>14</sup>C is greatly increased (by 200 per cent) immediately after a whole-body irradiation with 800 r. The synthesis of the globin in haemoglobin is increased in the spleen but not in the bone-marrow. As early as the second day after irradiation the synthesis of haemin in the bone-marrow falls to a very low level, and the synthesis of globin by the spleen returns to normal. The later course is interesting to follow, but is mainly concerned with the slow restoration of hematopoiesis. The most curious point is the complete dissociation between the synthesis of haemin, which is always increased in the spleen by x-rays, and that of globin, which increases immediately after irradiation, then falls to a very low level and does not rise again even after three weeks. The synthesis of globin seems to recover much more slowly than that of haemin<sup>87</sup>. A study of *Table III* shows that this investigation would have lost all its importance if the behaviour of the bone-

## PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

 Table III. Capacity of Bone-marrow and Spleen Homogenates to Synthesize Haemin and Globin (after RICHMOND et al.<sup>87</sup>) before and after Whole-body Irradiation

Series	Bone-marrow			Spleen		
	<i>W.</i> haemin × 10 <sup>-3</sup>	<i>W.</i> globin × 10 <sup>-3</sup>	<i>W.</i> haemin <i>W.</i> globin	<i>W.</i> haemin × 10 <sup>-3</sup>	<i>W.</i> globin × 10 <sup>-4</sup>	<i>W.</i> haemin <i>W.</i> globin
No radiation .	6.8	1.1	6.18	13.6	4.2	32.3
0 hours .	22.2	1.2	18.50	31.0	12.6	24.6
48 hours .	1.5	1.5	1.00	31.8	3.0	106.0
72 hours .	1.1	1.2	0.92	28.0	4.3	65.1
1 week .	0.6	0.4	1.50	53.0	0.9	588.9
2 weeks .	1.4	0.3	4.67	53.2	0.7	760.0
3 weeks .	—	0.2	—	44.5	0.3	1483.5
4 weeks .	18.7	0.5	37.40	—	—	—

Note: *W* is a factor used by the authors to express the synthetic activity.

marrow and spleen had not been observed immediately after irradiation.

The same workers also noticed an increase *in vitro* in the synthesis of saturated or unsaturated fatty acids by the bone-marrow of rabbits taken immediately after irradiation. In this case the tracer was acetate labelled with a <sup>14</sup>C atom in the β carbon (<sup>14</sup>CH<sub>3</sub>COONa)<sup>89</sup>. Table IV clearly shows that all the anabolic and catabolic activities of the bone-marrow are strongly stimulated by irradiation<sup>89</sup>.

Table IV. Synthetic Capacity of Bone-marrow before and after Whole-body Irradiation

Series	Saturated fatty acids		Unsaturated fatty acids		Haemin		O <sub>2</sub> uptake	<sup>14</sup> CO <sub>2</sub> activity
	<i>W*</i> × 10 <sup>-1</sup>	Per cent pre-radiation value	<i>W†</i> × 10 <sup>-1</sup>	Per cent pre-radiation value	10 <sup>4</sup> disint./min per mm per g wet wt bone-marrow	Per cent pre-radiation value	μl O <sub>2</sub> per g wet wt per 3 h	10 <sup>3</sup> disint./min/mm
No radiation .	6.31	—	5.50	—	1.22	—	300	5.66
0 hour	14.60	231	18.90	344	1.90	156	810	8.40
48 hours	5.96	108	—	—	0.31	25	280	4.70
72 hours	20.00	363	1.00	18	0.21	17	80	1.14
158 hours	3.21	58	15.60	283	0.05	4	280	0.18

\* *W* synthetic activity of saturated fatty acid from acetate.

† *W* synthetic activity of unsaturated fatty acid from acetate.

Nizet, Lambert, Herve and Bacq have attacked the same problem of haemoglobin synthesis *in vitro*, but avoided the artefacts in preparation, *i.e.* the homogenization carried out by the American authors. The reticulocytes, which are numerous in dogs made anaemic by bleeding, synthesize in the same way as bone-marrow

## INCREASED ENZYMIC AND SYNTHETIC ACTIVITY AFTER IRRADIATION

haemin and globin\* from labelled glycine *in vitro*<sup>90,138</sup>. No manipulation of the cells is required and it is only necessary to take samples of blood from the dog before and after 500 r of whole-body irradiation. An increased synthesis was found after irradiation which was of the same order of magnitude as that obtained by the Rochester team<sup>90</sup> (see Tables V and VI). Our technique enabled us to go

Table V. Effect of Irradiation on the Synthesis of Haemin by Reticulocytes of the Dog using <sup>14</sup>C labelled Glycine<sup>90</sup>

Experiment No.	61	87	102	108
Glycine —2— <sup>14</sup> C content d.p.m. <sup>†</sup> per ml of blood	222,000	222,000	74,000	92,500
Duration of incubation of the blood (h)	7	5	6	7

† Activity expressed as d.p.m. per 100 g of haemin isolated from the blood after incubation.

Blood from unirradiated dog . . .	14,200	1092	11,793	9,151
Blood irradiated in vitro (500 r) . . .	15,700	1554	27,035	—
"    "    "    " (2000 r) . . .	—	—	—	10,507
"    "    "    " (100,000 r) . . .	30,400	1237	19,884	—
Blood from dog which has received whole-body irradiation of 500 r . . .	28,000	1788	47,623	11,095
Non-irradiated erythrocytes + plasma from irradiated (500 r) dog . . .	—	—	19,180	13,088

Table VI. Effect of Irradiation on the Synthesis of Haemin by Reticulocytes of the Dog using <sup>14</sup>C labelled Phenylalanine<sup>138</sup>

Experiment No.	121	130	139
No. of red cells per ml of blood . . .	3,000,000	3,100,000	3,500,000
No. of reticulocytes per ml of blood . . .	210,000	295,000	210,000
Phenylalanine <sup>14</sup> C content d.p.m. <sup>†</sup> per ml of blood . . .	277,500	294,500	590,000
Duration of incubation of the blood (h) . . .	5	5	7

† Activity expressed as d.p.m. per 100 g of haemin isolated from the blood after incubation.

Blood from unirradiated dog . . .	851	999	703
Blood irradiated in vitro (500 r) . . .	1,244	—	—
"    "    "    " (20,000 r) . . .	1,019	1,234	722
Cells taken " " " before irradiation and plasma taken after irradiation . . .	929	1,097	—
Blood from dog which has received whole-body irradiation . . .	1,302	2,116	908

\* Probably because they contain small reserves of ribonucleic acid.

PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

further, and to observe (a) that the factor which increases haemin synthesis by the reticulocytes is, at least in part, dissolved in the plasma, for the synthesis of haemin by non-irradiated reticulocytes may be doubled by the addition of plasma from an irradiated dog; and (b) that an increase in haemin synthesis is also observed after irradiating heparinized blood *in vitro*, though much larger doses of x-rays are needed (2000 to 100,000 r).

Nucleated red corpuscles of fowls washed three times and suspended in saline solution (that is, in relatively unfavourable physio-

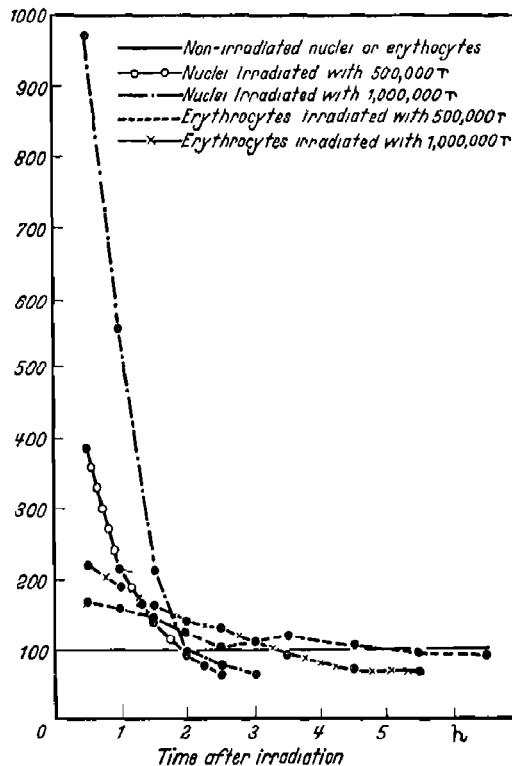


Figure 4. Effect of x-radiation on the respiration of nuclei of fowl erythrocytes and of whole erythrocytes. Oxygen consumption in the expil vessels expressed as per cent of oxygen consumption observed in control vessels at a given time<sup>120</sup>

logical conditions), at a concentration  $2 \times 10^6$  cells/mm<sup>3</sup>, double or treble their oxygen consumption immediately after a dose of 500,000 to 2,000,000 r of x-rays. The oxygen consumption returns to normal or falls below it about 6 hours after irradiation, when haemolysis of the corpuscles takes place<sup>118</sup>. If the nuclei are separated from the cytoplasm, and the effects of x-rays on whole corpuscles and on the isolated nuclei are compared, it is found that large doses cause a much greater increase in oxygen consumption in the isolated nuclei (Figure 4)<sup>120</sup>. The addition of albumin or

## INCREASED ENZYMIC AND SYNTHETIC ACTIVITY AFTER IRRADIATION

haemolysed blood to the suspension of nuclei protects them from the stimulating effect of x-rays. The cytoplasm apparently acts in the same way when whole corpuscles are irradiated<sup>120</sup>.

In ducks, the red corpuscles after total irradiation of the animal are said to consume less oxygen than do normal corpuscles<sup>119</sup>.

Alkaline phosphatase activity increases in spleen homogenates after irradiation, especially when the substrate is adenosinetriphosphate<sup>32, 33, 93</sup>. It is also increased in the plasma<sup>93</sup>. In the present state of our knowledge, we can only attribute this remarkable 'flaring up' of the haematopoietic cells to a change in the enzyme-substrate balance within the cells.

Whole-body irradiation of rats with x-rays causes a very distinct

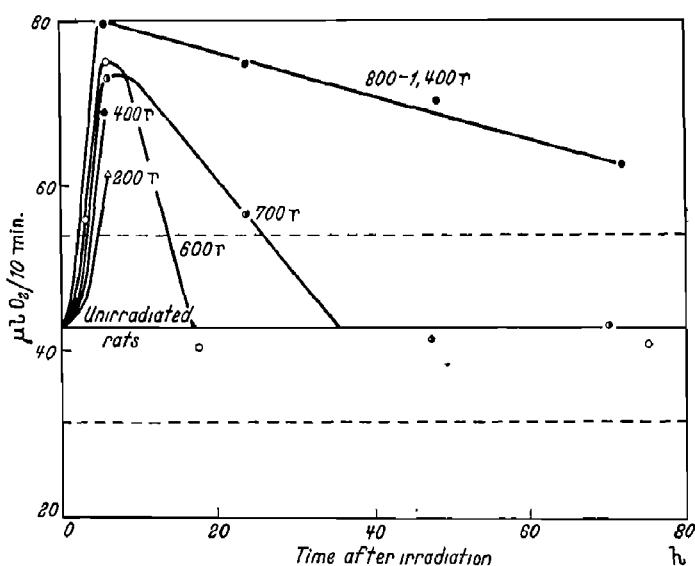


Figure 5. Endogenous respiration of rat liver homogenates after whole-body x-irradiation<sup>117</sup>

increase (Figure 5) in the oxygen consumption of the liver (homogenate), in spite of a diminution in the activity of choline oxydase. This effect is observed as early as 6 hours after a dose of 200 r. On the other hand, the oxygen consumption of the kidney, studied by the same technique, is not increased after irradiation, whereas the choline oxydase activity is greater 18 hours after a dose of 800 r than in controls<sup>117</sup>.

Some older experiments, often not confirmed, had already described increases in metabolism after irradiation, for instance in the skin of frogs, and in seeds and seedlings of radishes (see <sup>121</sup>).

PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

Except in the organs of vertebrates, however, the only recent confirmation of a considerable increase in metabolism is given by SUSSMAN<sup>121</sup> for potato tubers. The oxygen consumption is increased, on the average, by 500 per cent immediately after 10,000 to 400,000 r; the increase is less (200 to 300 per cent) after 1000 r and after very large doses (3,200,000 r) (Figure 6). After moderate doses the increase is very transitory.

There is also a smaller and less rapid increase in oxygen elimination, reaching its peak on the second day. The tuber blackens very quickly, confirming the observations of BACQ and HERVE<sup>64</sup> on a

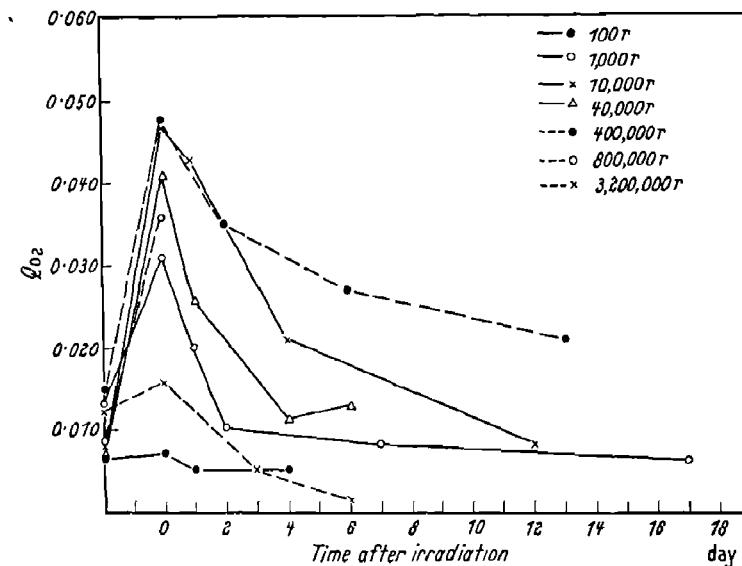


Figure 6. Effect of  $\gamma$ -radiation on oxygen uptake of potato tubers ( $Q_O_2$  calc. as mean of 6 tubers). The value of  $Q_O_2$  on the ordinate equals oxygen uptake before irradiation<sup>121</sup>

fungus containing tyrosinase, the blackening of the potato being brought about, like that of the fungus, by the reaction of a phenol (tyrosine, dopa etc.) with an oxydase which is not normally in contact with its substrate. It can be concluded from Sussman's experiment, as well as from ours, that one of the first effects of irradiation is the breaking down of intracellular barriers, putting enzymes in contact with a large quantity of substrate, which necessarily leads to increased metabolism.

The activity of cytochrome oxydase is not changed even by 3,200,000 r, whereas after this very large dose the activity of tyrosinase diminishes by about one-half, probably because of the

## INCREASED ENZYMIC AND SYNTHETIC ACTIVITY AFTER IRRADIATION

blackening, that is, because of the presence of quinones and melanin, which inhibit tyrosinase.

In this discussion we have carefully separated effects which are attributable to enzymatic adaptation from those attributable to the destruction of enzyme inhibitors by radiations. Nothing of this kind has been described in the bone-marrow and spleen. In Nizet and Bacq's investigations, when blood rich in reticulocytes was irradiated *in vitro*, any intervention by the adrenals was excluded; the phenomenon is cellular, and not dependent on mammalian organization. No inhibitor of haemoglobin synthesis has ever been recorded.

The importance of the localization, the strategic position and the variable degree of 'freedom' of the intracellular enzymes becomes more and more apparent. We are beginning to explore the possibilities of interaction between different structures. POTTER, LYLE and SCHNEIDER<sup>98</sup> for example, show that the rate at which the mitochondria carry out the oxidations of Krebs cycle is doubled by the addition of nuclear material equivalent to that present in the intact nuclei of the cells, although the nuclei alone are practically inactive. These observations support the hypothesis, discussed on p. 185, that changes in intercellular 'permeability' are among the important primary effects of ionizing radiations.

*Plasma enzymes or tissue extracts*—Small doses of x-rays (25 or 50 r) greatly increase the peptidase activity of plasma taken from dogs 24 hours after irradiation. This increase is especially pronounced (+400 per cent) if benzoyl argininamide is used as a substrate<sup>94</sup>; MILLER and GATES suggest this as a biochemical test to show that the body as a whole has been exposed to a small dose of ionizing radiations<sup>94</sup>.

EULER and HEVESY<sup>95</sup> isolated the catalase of Jensen's sarcoma and found the activity to be increased by 53 per cent after 3000 r. FORSSBERG has observed a less marked activation of catalase *in vivo* by x-rays (see reference 91).

*Increased incorporation of isotopically labelled tracers\**—The uptake of <sup>131</sup>I by the thyroid is increased both by irradiation of the gland and of the whole body<sup>81, 82, 83</sup>. Incorporation of <sup>32</sup>P in the ribonucleic acid of the cytoplasm of the liver cells is increased<sup>84</sup>, and this confirms MITCHELL's<sup>85, 86</sup> finding that after irradiation *in vivo* of cancer tissue there is in the cytoplasm a significant increase in the u.v. absorption at a wavelength characteristic of the nucleic acids. The incorporation of <sup>14</sup>C from labelled glycine in the brain of rats is also

---

\* See HEVESY<sup>2, 44</sup> for the limitations as regards <sup>14</sup>C and <sup>32</sup>P attendant on this method.

PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

increased if the glycine is injected 48 hours after a whole-body irradiation<sup>92</sup>.

SHERMAN and FORSSBERG<sup>137</sup> have made a very complete study of the incorporation of  $^{32}\text{P}$  in the liver of the mouse and of the changes brought about by a whole-body dose of 800 r of x-rays. The amount of radioactive phosphorus found in the liver following injection in the pleura in the form of orthophosphate is increased by 40 per cent if it is injected immediately after irradiation. As the animal is sacrificed 5 minutes after the injection, this finding indicates that there is a strong increase in permeability to phosphate ions: The effect of radiation becomes less marked as the time interval is increased (Figure 7). The quantity of  $^{32}\text{P}$  incorporated in the labile phosphate fraction in the trichloracetic acid tract behaves in

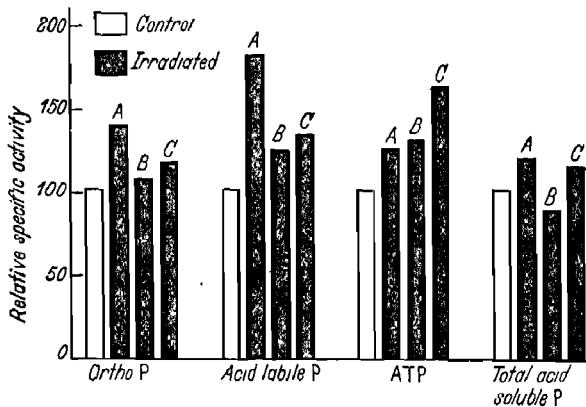


Figure 7. Relative specific activity of ortho P, acid-labile P, ATP, and total acid-soluble P from mouse liver irradiated *in vivo* with 800 r of x-rays. A, animals injected with  $^{32}\text{P}$  immediately after irradiation. B, animals injected with  $^{32}\text{P}$  60 min after irradiation. C, animals injected with  $^{32}\text{P}$  24 h after irradiation<sup>137</sup>

the same way; the increase immediately after irradiation is even more marked. The activity of ATP is also increased, especially 24 hours after irradiation. The variations in activity of the total acid-soluble phosphorus are less pronounced and less significant.

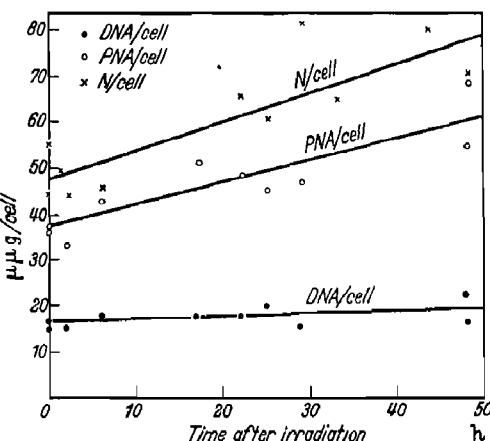
FORSSBERG and HEVESY<sup>137</sup> show that after irradiation the  $^{32}\text{P}$  passes more quickly both through the hepatic capillaries and the cellular membranes. The  $^{32}\text{P}$  is incorporated in the organic compounds and is consequently lost to the pool of ions which exchange constantly through the cellular membrane. This interpretation is confirmed by the fact that if the liver is irradiated *in vitro*, the incorporation of  $^{32}\text{P}$  in the slices of tissue is not modified. In the muscle (irradiated *in vivo*) the rate of exchange of  $^{32}\text{P}$  and its incorporation are slightly decreased by an irradiation with 800 r.

## INCREASED ENZYMIC AND SYNTHETIC ACTIVITY AFTER IRRADIATION

These researches show: (i) that the liver is a radiosensitive organ, (ii) that its vascular system must be intact if certain effects of the ionizing radiations are to become apparent, (iii) that the results obtained with one organ cannot, at least for mammals, be extrapolated to other tissues.

FORSSBERG and KLEIN have made an excellent study of the biochemical reactions of the Ehrlich ascites tumour to x-rays<sup>127, 128</sup>. This material is particularly suitable for biochemical researches since it consists of isolated cells suspended in the peritoneum which can be inoculated into mice. These cells are not contaminated by blood vessels or connective tissue and the increase in the number of cells can be followed quantitatively. The first investigation of these authors<sup>127</sup> showed that 1250 r, although stopping mitosis for

*Figure 8. Increase of the average amount of RNA, DNA, and total N per cell after irradiation<sup>127</sup> with 1250 r*



16 hours, did not inhibit growth or the synthesis of proteins and RNA. The synthesis of DNA, however, is greatly reduced. Each cell (nucleus and protoplasm) doubles its volume in 48 hours, but instead of multiplying like normal cells, the irradiated cells increase in volume. There is clearly a correlation between mitosis arrest and inhibition of DNA synthesis (see *Figure 8*).

These results were confirmed quantitatively by the same authors<sup>128</sup> when studying the influence of irradiation on the incorporation of <sup>14</sup>C of the glycine in the various constituents of the ascites tumour. In the first two hours after irradiation many rapid changes occur and the results obtained cannot be expressed by straight lines or smooth curves. Thirty-five minutes after irradiation the total quantity of ATP (+ADP)\* is increased by 27 per cent, whereas the activity

\* ADP = adenosine diphosphoric acid brought about by the partial hydrolysis of ATP : *i.e.* ATP → ADP + phosphate.

#### PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

(incorporation of  $^{14}\text{C}$ ) is 15 per cent less than in the controls; 80 minutes after irradiation the irradiated values are the same as those of the controls. During the first hour after irradiation the amount of ATP used up is decreased while its synthesis is unaffected. The incorporation of  $^{14}\text{C}$  into the purines of DNA and RNA is slowed down considerably during the two hours following irradiation, but its incorporation in proteins is only reduced by 15 per cent during the first hour. Incorporation in the lipids is slowed down in the first 35 minutes, is increased by 60 per cent after 80 minutes and becomes normal at 120 minutes. Although the total synthesis of DNA is reduced to one-third by irradiation, the incorporation of  $^{14}\text{C}$  in DNA in the irradiated cells is 70 to 75 per cent that of an identical non-irradiated cell population. It would appear that the synthesis of the purines for DNA is less affected by irradiation than the synthesis of the complete macromolecule. These results are in agreement with the suggestion that irradiation essentially affects the assembly of various molecules (bases, pentose and phosphate) which form DNA while the antagonists, the folic acid especially, inhibit the synthesis of the purine and pyrimidinic bases<sup>139</sup>.

#### INHIBITION OF ISOLATED ENZYME SYSTEMS *IN VIVO*

It cannot be sufficiently emphasized that impure enzymes or enzymes *in vivo* are highly resistant to irradiation, whereas pure aqueous solutions are sensitive *in vitro*<sup>30, 91, 107</sup>. Exposure of whole cells of the ciliated protozoon *Tetrahymena geleii* or *T. pyriformis* to 300,000 or 600,000 r of x-radiation has very little effect on oxidative or hydrolytic enzyme activity of the cells. However, if cell-free homogenates are irradiated, then the activities of these enzymes diminish considerably<sup>134, 135</sup>. The enzymes may be protected *in vivo* by their localization on intracellular structures.

The 'pseudo' cholinesterase activity (or non-specific cholinesterase) of muscle and intestinal mucosa of rats was diminished by about 50 per cent 48 hours after a whole-body irradiation of 1000 r; but after 24 hours there is little diminution. There is no change in the level of true (or specific) cholinesterase, which physiologists regard as more important. The response of smooth muscle to acetylcholine is increased within the first 24 hours in the colon, and after 48 hours in the jejunum and ileum. The difference between the two cholinesterases may be due to the fact that the source of pseudo-cholinesterase appears to be the liver, whereas true cholinesterase originates elsewhere, and may be synthesized locally<sup>105</sup>. These observations were confirmed by CONARD<sup>106</sup>. There are also

#### INHIBITION OF ISOLATED ENZYME SYSTEMS *IN VIVO*

changes in the plasma pseudo-cholinesterase of guinea-pigs, but this must be interpreted cautiously because fasting rapidly lowers the concentration of this enzyme<sup>1</sup>.

The hydroquinone oxydase in the ova of a grasshopper (*Melanoplus differentialis*) is diminished for 2 days after irradiation with 25,000 r after which it gradually increases (to 250 per cent of normal activity), while the sulphhydryl groups disappear<sup>24</sup>.

Catalase activity is claimed to be decreased appreciably (20 per cent) in the liver of mice 24 hours after irradiation<sup>108, 109</sup> to return to normal at about 48 hours, and to fall finally to a very low value on the fifth to seventh day. This may be an indirect effect, for the Japanese authors have observed inactivation of this enzyme in mice after injection of rabbit's serum taken 1 to 5 days after irradiation<sup>110</sup>. These findings are not easy to reconcile with those of EULER and HEVESY<sup>95</sup> as it would be necessary to postulate either that large doses of x-rays can activate where small doses inactivate, or that the behaviour of Jensen's rat sarcoma is opposite to that of the liver.

#### SUMMARY

To sum up, it is surprising to find that, except during the last days of life, little change in general metabolism is caused by irradiation with the minimum 100 per cent lethal dose of x-rays or less. Carbohydrates, fats and proteins are still absorbed, in spite of the precarious anatomical condition of the intestines. The disturbances which are observed are of a trivial type and can often be regarded as secondary to more or less marked anorexia, the loss of water and electrolytes resulting from diarrhoea, vomiting, or disturbances in capillary permeability. There are endocrine disturbances in irradiated mammals, but they do not explain what happens during the first hours after irradiation. Inhibition of the sulphhydryl enzymes has not been confirmed *in vivo*.

The few points about which there seems to be agreement are: the slowing down of desoxyribonucleic acid synthesis; the *early* increase in catabolic and anabolic processes in the bone-marrow, spleen and reticulocytes.

An appreciable number of investigators are impressed by two general hypotheses: (i) The disturbance in 'osmotic' balance in the various intracellular structures (nuclei, nucleoli, mitochondria, microsomes etc). (ii) The uncoupling of oxidative phosphorylation; but as all who have studied this problem agree, we are far from being able to explain in biochemical terms why some cells are much more radiosensitive than others. There is, however, no doubt that

## PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

the concept of a biochemical lesion is valid; that radiation sickness is the result of a series of biochemical lesions is proved by many definite facts. GRAY<sup>132</sup> has stressed that the energy absorbed by a cell during irradiation of a few hundreds of roentgens, sufficient to kill many cells, is extremely small and can only bring about a chemical change in a few hundred molecules per cubic micron ( $\mu^3 = 10^{-12}$  ml) of tissue. The primary effect must therefore be discrete, such as the disruption of a few macromolecules at a vital centre.

### REFERENCES

- <sup>1</sup> ORD, M. G. and STOCKEN, L. A., *Physiol. Rev.*, 1953, **33**, 356
- <sup>2</sup> HEVESY, G., 'Ionizing radiation and cellular metabolism', *Oberlin Symp. Radiobiol.*, Wiley, New York, 1952, p. 189
- <sup>3</sup> SMITH, F., BUDDINGTON, W. G. and GRENNAN, M. M., *Proc. Soc. exp. Biol. Med.*, 1952, **81**, 140
- <sup>4</sup> SMITH, D. E., TYREE, E. B., PATT, H. M. and JACKSON, E., *ibid.*, 1951, **78**, 774
- <sup>5</sup> MOLE, R. H., *Quart. J. exp. Physiol.*, 1953, **38**, 69
- <sup>6</sup> KIRSCHNER, L. B., PROSSER, C. L. and QUASTLER, H., *Proc. Soc. exp. Biol. Med.*, 1949, **71**, 463
- <sup>7</sup> HEVESY, G. and FORSSBERG, A., *Nature, Lond.*, 1951, **168**, 692
- <sup>8</sup> LEVY, B. and RUGH, R., *Proc. Soc. exp. Biol. Med.*, 1953, **82**, 223
- <sup>9</sup> ROSS, M. H. and ELY, J. O., *J. cell. comp. Physiol.*, 1951, **37**, 163
- <sup>10</sup> FISCHER, P., *Arch. int. Physiol.*, 1954, **62**, 134
- <sup>11</sup> LOURAU, M. and LARTIGUE, O., *J. Physiol., Paris*, 1951, **43**, 593
- <sup>12</sup> MCKEE, R. W., *Fed. Proc.*, 1951, **11**, 256
- <sup>13</sup> LOURAU-PITRES, M., *Arch. Kem.*, 1954, in press
- <sup>14</sup> BARRON, E. S. G. and FLOOD, V., *J. gen. Physiol.*, 1950, **33**, 229
- <sup>15</sup> BARRON, E. S. G., DICKMAN, S., MUNTZ, J. A. and SINGER, T. P., *ibid.*, 1949, **32**, 537
- <sup>16</sup> BARRON, E. S. G. and DICKMANS, S., *ibid.*, 1949, **32**, 595
- <sup>17</sup> BARRON, E. S. G. and SEKI, S. L., *ibid.*, 1952, **35**, 865
- <sup>18</sup> FISCHER, P., DE LANDTSHEER, L. and LECOMTE, J., *Bull. Soc. Chim. biol.*, 1950, **32**, 1009
- <sup>19</sup> MASSART, L., personal communication, 1953
- <sup>20</sup> SHACTER, B., SUPPLEE, H. and ENTENMAN, C., *Amer. J. Physiol.*, 1952, **169**, 499
- <sup>21</sup> PETERSON, R. D., BEATTY, C. H. and WEST, E. S., *Proc. Soc. exp. Biol. Med.*, 1951, **77**, 747
- <sup>22</sup> LANGENDORFF, H., *Strahlentherapie*, 1950, **83**, 33
- <sup>23</sup> FREDERIC, J., *Arch. Biol.*, 1949, **60**, 79
- <sup>24</sup> TAHMISIAN, T. N. and ADAMSON, D. M., *J. exp. Zool.*, 1950, **115**, 379
- <sup>25</sup> PATT, H. M., *Physiol. Rev.*, 1953, **33**, 61
- <sup>26</sup> SHACTER, B., *Cancer Res.*, 1951, **2**, 277

## REFERENCES

- <sup>27</sup> LIPMANN, F., *Bact. Rev.*, 1953, **17**, 1  
<sup>28</sup> THOMSON, J. F. and MIKUTA, E. T., *Argonne nat. Lab. Quart. Rep.*, 4932, 1953; *Proc. Soc. exp. Biol. Med.*, 1954, **86**, 487  
<sup>29</sup> BACQ, Z. M. and HEUSGHEM, C., unpublished observations  
<sup>30</sup> LE MAY, M., *Proc. Soc. exp. Biol. Med.*, 1951, **77**, 337  
<sup>31</sup> FISCHER, M. A., COULTER, E. P. and COSTELLO, M. J., *ibid.*, 1953, **83**, 266  
<sup>32</sup> ASHWELL, G. and HICKMAN, J., *ibid.*, 1952, **80**, 407  
<sup>33</sup> MAXWELL, E. and ASHWELL, G., *Arch. Bioch.*, 1953, **43**, 389  
<sup>34</sup> BARRON, E. S. G., *Oberlin Symp. Radiobiol.*, Wiley, New York, 1952, p. 216  
<sup>35</sup> THOMSON, J. F., TOURTELLOTTE, W. W. and CARTTAR, M. S., *Proc. Soc. exp. Biol. Med.*, 1952, **80**, 268  
<sup>36</sup> ELLINGER, F., *Radiology*, 1945, **44**, 241  
<sup>37</sup> INGRAM, M., MASON, W. B., WHIPPLE, G. H. and HOWLAND, J. W., *Univ. Rochester Atom. Energy Proj.*, UR-196, 1952  
<sup>38</sup> DOBROVOLSKAIA-ZAVADSKAIA, N. and MACCHI, L., *J. Radiol. Electrol.*, 1952, **33**, 237  
<sup>39</sup> DUBOIS, K. P., COCHRAN, K. W. and DOULL, J., *Proc. Soc. exp. Biol. Med.*, 1951, **76**, 422  
<sup>40</sup> PETERS, R. A., WAKELIN, R. W. and BUFFA, P., *Proc. roy. Soc.*, 1953, **B140**, 497  
<sup>41</sup> POTTER, V. R., BUSCH, H. and BOTHWELL, J., *Proc. Soc. exp. Biol. Med.*, 1951, **76**, 38  
<sup>42</sup> BEAULIEU, M. M. and DALLEMAGNE, M. J., *Bull. Soc. Chim. biol.*, 1953, **35**, 969  
<sup>43</sup> HICKMAN, J. and ASHWELL, G., *Fed. Proc.*, 1953, **12**, 66  
<sup>44</sup> HEVESY, G. and DREYFUS, G., *Ark. Kemi*, 1952, **4**, 337  
<sup>45</sup> THOMPSON, H. E. and STEADMAN, L. T., *Univ. Rochester Atom. Energy Proj.*, UR-152, 1951  
<sup>46</sup> STEADMAN, L. T. and GRIMALDI, A. J., *ibid.*, UR-205, 1952  
<sup>47</sup> FURTH, F. W., WATSON, R. and COULTER, M. P., *ibid.*, UR-164, 1951  
<sup>48</sup> MEAD, J. F., *Science*, 1952, **115**, 470  
<sup>49</sup> DUBOULZ, P., DUMAS, J. and VIGNE, J., *C.R. Soc. Biol.*, 1950, **144**, 1080  
<sup>50</sup> BACQ, Z. M., *Experientia*, 1951, **7**, 11  
<sup>51</sup> DUBOULZ, P. and DUMAS, J., *C.R. Acad. Sci., Paris*, 1952, **234**, 2575  
<sup>52</sup> COOKE, A. R., *Science*, 1953, **117**, 588  
<sup>53</sup> DUBOULZ, P. and FONDARAI, J., *Bull. Soc. Chim. bul.*, 1953, **35**, 819  
<sup>54</sup> BACQ, Z. M., BURG, C., CHEVALLIER, A. and HEUSGHEM, C., *J. Physiol., Paris*, 1951, **43**, 640  
<sup>55</sup> HORGAN, V. J. and PHILPOT, J. St. L., *Trans. Faraday Soc.*, 1953, **49**, 324  
<sup>56</sup> BACQ, Z. M. and HERVE, A., *C.R. Soc. Biol.*, 1949, **143**, 1158  
<sup>57</sup> DUSTIN, P. and GOMPEL, C., *ibid.*, 1949, **143**, 874  
<sup>58</sup> ALPER, T., *Nature, Lond.*, 1953, **172**, 957  
<sup>59</sup> RONDONI, P. and CUDKOWICZ, G., *Experientia*, 1953, **9**, 348  
<sup>60</sup> ROSENTHAL, R. L., *Univ. Calif. Rad. Lab. Rep.*, UCRL-273, 1949  
<sup>61</sup> STEADMAN, L. T. and THOMPSON, H. E., *Univ. Rochester Atom. Energy Proj.*, UR-103, 1950  
<sup>62</sup> STEADMAN, L. T. and GRIMALDI, A. J., *ibid.*, UR-227, 1952

PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

- <sup>63</sup> LOISELEUR, J. and VELLEY, G., *C.R. Acad. Sci., Paris*, 1950, **231**, 529
- <sup>64</sup> BACQ, Z. M. and HERVE, A., *Bull. Acad. Méd. Belg.*, 1952, 6th series, **17**, 13
- <sup>65</sup> LOW-BEER, A., *Strahlentherapie*, 1933, **46**, 469
- <sup>66</sup> LEBLOND, C. P. and SEGAL, G., *Amer. J. Roentgenol.*, 1942, **47**, 302
- <sup>67</sup> CHEVALLIER, A., BURG, C. and SPEHLER, H., *C.R. Soc. Biol.*, 1953, **147**, 497
- <sup>68</sup> ENTENMAN, C. and WEINMAN, E. O., *Fed. Proc.*, 1952, **11**, 44
- <sup>69</sup> STEANER, S. P., BRUES, A. M. and CHRISTIAN, E. J. B., *Nucl. Sci. Abstr.*, 1951, **5**, 1723, 2322
- <sup>70</sup> HERBERT, P. and MOLE, R. H., cited by Ord and Stocken<sup>1</sup>
- <sup>71</sup> GJESSING, E. C. and CHANUTIN, A., *Arch. Biochem.*, 1950, **27**, 191
- <sup>72</sup> KOHN, H. T., *Amer. J. Physiol.*, 1950, **162**, 703
- <sup>73</sup> KNOX, W. E., *Brit. J. exp. Path.*, 1951, **32**, 462
- <sup>74</sup> THOMSON, J. F. and MIKUTA, E. T., *Argonne nat. Lab.*, 1952, ANK-4794, p. 140; ANL-4840, p. 80; ANL-4932, p. 59
- <sup>75</sup> STEANER, S. P., *Argonne nat. Lab., Mon. Rep.*, ANL-5086, 1953, p. 9
- <sup>76</sup> FEINSTEIN, R. N. and BALLIN, J. C., *Proc. Soc. exp. Biol. Med.*, 1953, **83**, 10
- <sup>77</sup> FEINSTEIN, R. N. and BALLIN, J. C., *ibid.*, 1953, **83**, 6
- <sup>78</sup> ABBERHALDEN, R., *Strahlentherapie*, 1940, **68**, 17
- <sup>79</sup> LAMERTON, L. F., ELSON, L. A. and CHRISTENSEN, W. R., *Brit. J. Radiol.*, 1953, **26**, 510
- <sup>80</sup> BAIR, W. G. and STANNARD, J. N., *Univ. Rochester Atom. Energy Proj.*, UR-189, 1951
- <sup>81</sup> HURSH, J. B., VALKENBURG, P. A. VAN and MOHENNY, J. B., *J. Radiol.*, 1951, **56**, 411
- <sup>82</sup> EVANS, T. C., CLARKE, G. and SOBEL, E., *Anat. Rec.*, 1947, **99**, 577
- <sup>83</sup> BOTKIN, A. L., PRAYTOR, E. H., AUSTING, M. E. and JENSEN, J., *Nucl. Sci. Abstr.*, 1952, **6**, 35
- <sup>84</sup> PAYNE, A. H., KELLY, L. S. and ENTENMAN, C., *Proc. Soc. exp. Biol. Med.*, 1952, **81**, 698
- <sup>85</sup> MITCHELL, J. S., *Nature, Lond.*, 1940, **146**, 272
- <sup>86</sup> MITCHELL, J. S., *Brit. J. exp. Path.*, 1942, **23**, 285
- <sup>87</sup> RICHMOND, J. E., ALTMAN, K. I. and SALOMON, K., *J. biol. Chem.*, 1951, **190**, 817
- <sup>88</sup> RICHMOND, J. E., ALTMAN, K. I. and SALOMON, K., *Science*, 1951, **113**, 407
- <sup>89</sup> ALTMAN, K. I., RICHMOND, J. E. and SALOMON, K., *Bioch. Biophys. Acta*, 1951, **7**, 460
- <sup>90</sup> NIZET, A., LAMBERT, S. and BACQ, Z. M., *Arch. int. Physiol.*, 1954, **62**, 129
- <sup>91</sup> EULER, H. von and HAHN, L., *Acta Radiol.*, 1946, **27**, 269
- <sup>92</sup> ALTMAN, K. I., CASARETT, G. W., NOONAN, T. R. and SALOMON, K., *Fed. Proc.*, 1949, **8**, 349
- <sup>93</sup> LUDEWIG, S. and CHANUTIN, A., *Arch. Biochem.*, 1950, **29**, 441
- <sup>94</sup> MILLER, L. L. and GATES, E., *Univ. Rochester Atom. Energy Proj.*, 1949, UR-96, p. 13
- <sup>95</sup> EULER, H. von and HEVESY, G., *Biol. Medd., Kbh.*, 1942, **17**, No. 8

## REFERENCES

- 96 ELLINGER, F., *Atomic Medicine*, Williams and Wilkins, Baltimore, 2nd edn, 1953, p. 85  
 97 ANCEL, P. and VINTERBERGER, P., *C.R. Soc. Biol.*, 1924, **91**, 606  
 98 POTTER, V. R., LYLE, G. G. and SCHNEIDER, W. C., *J. biol. Chem.*, 1951, **190**, 293  
 99 VOLKIN, E. and KOHN, H. T., *Arch. Biochem.*, 1951, **30**, 326  
 100 KOHN, H. T., *Amer. J. Physiol.*, 1950, **162**, 703  
 101 HOLMES, B. E. and MEE, L. K., *Brit. J. Radiol.*, 1952, **25**, 273  
 102 KOHN, H. T., *J. Immunol.*, 1951, **66**, 525  
 103 SUPPLEE, H., HAUSCHILD, J. D. and ENTENMAN, C., *Amer. J. Physiol.*, 1952, **169**, 482  
 104 KOHN, H. T., *ibid.*, 1951, **165**, 27, 43  
 105 BURN, J. H., KORDIK, P. and MOLE, R. H., *Brit. J. Pharmacol.*, 1952, **7**, 58  
 106 CONARD, R. A., *Amer. J. Physiol.*, 1952, **170**, 418  
 107 BYERS, S. O., TYTELL, A. A. and LOGAN, M. A., *Arch. Biochem.*, 1949, **22**, 66  
 108 FEINSTEIN, R. M., BUTLER, C. L. and HENDLEY, D. D., *Science*, 1950, **111**, 149  
 109 MORI, K., MOMOKI, S. and ITO, H., *Igaku to Seibuts.*, 1951, **18**, 303  
 110 MORI, K., MOMOKI, S. and ITO, H., *ibid.*, 1951, **19**, 95  
 111 ZAHL, P. A. and ALBAUM, H. G., *Proc. Soc. exp. Biol. Med.*, 1951, **77**, 388  
 112 HEVESY, G., *J. Chim. phys.*, 1951, **48**, 275  
 113 MARINELLI, L. D. and KENNEY, J. M., *Radiology*, 1941, **37**, 691  
 114 JONES, H. B., *Symp. Radiobiology* (ed. J. J. Nickson), Wiley, New York, 1952, p. 118  
 115 HEVESY, G., *Adv. Biol. méd. Phys.*, 1948, **1**, 409  
 116 BRACHET, J., *Actual. Bioch.* No. 16, Desoer, Liège; Masson, Paris, 1952  
 117 KUNCKEL, H. O. and PHILLIPS, P. H., *Arch. Biochem.*, 1952, **37**, 366  
 118 FRANKENTHAL, L. and BACK, A., *Bioch. J.*, 1944, **38**, 351  
 119 KLEIN, J. R., *Fed. Proc.*, 1952, **11**, 240  
 120 BACK, A. and BLOCH-FRANKENTHAL, L., *Proc. Soc. exp. Biol. Med.*, 1947, **66**, 366  
 121 SUSSMAN, A. S., *J. cell. comp. Physiol.*, 1953, **42**, 273  
 122 PATT, H. M., *Annu. Rev. Physiol.*, 1954, **16**, 51  
 123 BETZ, H., *C.R. Soc. Biol.*, 1952, **146**, 315, 318, 325  
 124 KRETCHMAR, A. L., GOMBERG, H. J., WEYANT, D. E. and BETHELL, F. H., *Endocrinology*, 1952, **51**, 59  
 125 COMSA, J. and GROS, C. M., *Nature, Lond.*, 1954, **173**, 307  
 126 COMSA, J., *Ann. Endocrin.*, 1953, **13**, 931  
 127 KLEIN, G. and FORSSBERG, A., *Exp. Cell Res.*, 1954, **6**, 211  
 128 FORSSBERG, A. and KLEIN, G., *Exp. Cell Res.*, in press  
 129 HALEY, T. H., McCULLOCH, E. F. and MCCORMIK, W. G., *Science*, 1954, **119**, 126  
 130 FLORSHEIM, W., DOERNBACH, C. and MORTON, M. E., *Proc. Soc. exp. Biol. Med.*, 1952, **81**, 121  
 131 MIKAELSON, K. and HALVAREN, H., *Physiol. Plant.*, 1953, **6**, 873

PATHOLOGICAL BIOCHEMISTRY OF IRRADIATED LIVING ORGANISMS

- <sup>132</sup> GRAY, L. H., *Rad. Res.*, 1954, **1**, 180
- <sup>133</sup> HEVESY, G., *Acta physiol. scand.*, 1953, **30**, 90
- <sup>134</sup> EICHEL, H. J. and ROTH, J. S., *Biol. Bull.*, 1953, **104**, 351
- <sup>135</sup> EICHEL, H. J. and ROTH, J. S., *ibid.*, 1953, **105**, 373
- <sup>136</sup> BRUES, A. M. and PATT, H. M., *Physiol. Rev.*, 1953, **33**, 85
- <sup>137</sup> SHERMAN, F. G. and FORSSBERG, A., *Arch. Biochem.*, 1954, **48**, 293
- <sup>138</sup> NIZET, A. and HERVE, A., *Symp. Radiobiol.*, Liège, Butterworths, London, 1955
- <sup>139</sup> LAJTA, L. G. and OLIVER, R., *ibid.*
- <sup>140</sup> VAN BEKKUM, D. W., *Symp. Radiobiol.*, Liège, Butterworths, London, 1955
- <sup>141</sup> DUBOIS, K. P. and PATERSEN, D. F., *Amer. J. Physiol.*, 1954, **176**, 282
- <sup>142</sup> ERRERA, M., *Symp. Radiobiol.*, Liège, Butterworths, London, 1955
- <sup>143</sup> CHEVALLIER, A. and BURG, C., *ibid.*

## PROCESSES OF RESTORATION AFTER IRRADIATION

ONE of the great problems in theoretical and practical radiobiology is whether it is possible to influence the restorative processes which tend to re-establish normal physiological conditions after irradiation. Its theoretical importance lies in the fact that its solution may indicate what happens during irradiation, and from the practical point of view the answer will provide the scientific basis for the treatment of irradiation victims.

In theory, several kinds of restoration are conceivable <sup>5, 9</sup>: (i) Neutralization of the *primary* effects produced at the molecular level by the free radicals or by direct action. Although in model experiments it has proved possible to repair damaged molecules by a transfer reaction (see p. 131) it is highly improbable that this can be done *in vivo* after irradiation, since the damaged molecules rapidly undergo further irreversible changes. (ii) Neutralization of the *secondary* effects. The cells resynthesize molecules which irradiation has made unusable, and the question is whether it is possible to accelerate this synthesis, which is related to *cicatrization* at the tissue level (*e.g.* cutaneous burns), or at the cellular level. These secondary effects are many and varied, and can be modified by various methods after irradiation.

The recent literature is unequivocal: it is as easy to accelerate restoration after u.v. irradiation as it is difficult to influence lesions due to ionizing radiations if action can only be taken after irradiation.

With regard to u.v. light, LATARJET's investigations<sup>1</sup> have shown in micro-organisms how great is the importance of restoration by catalase (which destroys the peroxides formed in the system) and by visible and infra-red light—*photorestoration*. This is a general phenomenon, and is also observed if the ova or spermatozoa of certain echinoderms (*Arbacia punctulata*) are used instead of micro-organisms<sup>2</sup>. However, if the same methods of restoration are tried on the same isolated cells or organisms after irradiation with x-rays, the results are generally negative<sup>1, 2\*</sup>. In this respect the behaviour of ionizing radiations is quite different from that of u.v. radiations. BLUM

---

\* The only positive response of lesions produced by x-rays to visible light was reported by LATARJET for a strain of lysogenic *B. megatherium*<sup>9</sup>.

#### PROCESSES OF RESTORATION AFTER IRRADIATION

*et al.*<sup>2</sup> suppose that the principal lesion in the ova of *Arbacia* caused by u.v. as well as by x-rays is in the nucleoproteins of the nucleus whereas photorestoration takes place in the cytoplasm.

Two kinds of biological material, micro-organisms and the eggs of the silkworm, *Bombyx mori*, have been carefully studied to determine restoration process after ionizing radiation. The skin erythema of man is too complicated a test for quantitative studies, though it has given excellent results in the hands of HOLTHUSEN<sup>6</sup>. The reactions of the eggs of *B. mori* are constant and can be studied accurately<sup>5</sup>. The criterion used is the hatching of the eggs. By fractionating the dose given it was possible to show that restorative processes appear if the eggs are incubated at 21° after irradiation. If the eggs are kept in the refrigerator between two irradiations\* the effects of the two irradiations are strictly cumulative. About 1000 r (two-thirds of the total dose) is the optimal dose for the demonstration of the restorative processes.

The restorative potential of a tissue can be gauged by irradiating it continuously until a given effect is obtained (*e.g.* cessation of mitosis, cutaneous erythema). With irradiation of moderate intensity the effect is proportional to the total dose, *i.e.* it is independent of the duration and intensity of the irradiation; but with low intensities it is necessary to increase the total dose, that is, below a certain intensity (termed the critical intensity), restoration takes place in spite of the continuance of irradiation. With extremely low intensities ( $1 \times 10^{-5}$  to  $10^{-6}$  r/sec for human skin) the radiation lesion does not appear, and it may be said that the lesions are repaired as they are produced (see also p. 271).

HOLLAENDER and his collaborators<sup>7</sup> observed that if *E. coli* is incubated at a suboptimal temperature† after irradiation, partial restoration takes place, judging from the percentage of cells capable of forming colonies after transplantation. The temperature which allows the maximum of restoration when bacteria are incubated after irradiation is different for every strain, and is characteristic of that strain. For example, the best temperature for restoration in *E. coli* is 18°; there is much less restoration at 30° and at 6°. The presence of oxygen is necessary. The incidence of mutations is also much lower if irradiated *E. coli* are incubated at the optimal temperature for restoration (see *Figure 1*).

\* The second irradiation makes it possible to ascertain from control experiments the state of radiosensitivity at various times after the first irradiation. These restorative processes are practically complete after 8 hours; they neutralize at most three-quarters of the effect of the first irradiation.

† A suboptimal temperature is one which is lower than that which allows maximum growth of the normal strain, but still allows some enzymatic activity.

#### PROCESSES OF RESTORATION AFTER IRRADIATION

HOLLAENDER<sup>7</sup> found a substance in the spleen of mammals, which is soluble in acids and alcohols, fairly resistant to heat, and which promotes restoration in irradiated bacteria. The chemical nature of this substance is unknown, but a very active factor which has a molecular weight of 20,000 to 40,000, and which is neither a nucleic acid nor a known enzyme, has been isolated by paper chromatography (1 g from 200 kg of spleen). It promotes restoration in bacteria after irradiation with x-rays, but not with u.v. A factor

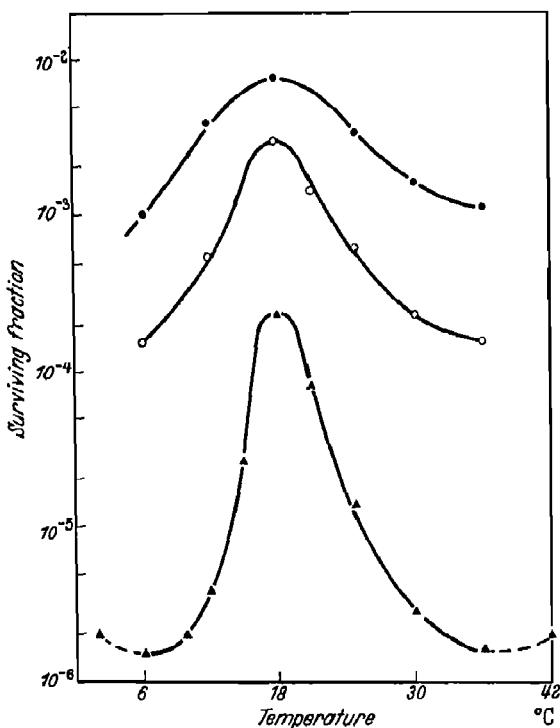


Figure 1. Survival of *E. coli* B/r as a function of temperature after doses of x-rays as follows: ● 40 kr; ○ 60 kr; △ 80 kr<sup>11</sup>

which is apparently identical with it has been found in yeast extracts, and liver also contains a small amount. It is not found if the spleen has been irradiated before its removal.

This splenic factor which influences the processes of restoration in bacteria is probably not identical with that which exists in homogenates of spleen and stimulates regeneration of the haematopoietic system in irradiated rodents (see p. 339). The injection of these homogenates (or the abdominal implantation of the spleen of

## PROCESSES OF RESTORATION AFTER IRRADIATION

new-born animals) is the only action which invariably promotes restoration in mammals when carried out after irradiation.

Warm-blooded animals are not very suitable for these studies on restoration, since it is mainly by varying the temperature after irradiation that the processes of restoration can be influenced in silkworm eggs and in bacteria.

MAISIN and his collaborators<sup>3</sup> obtained very favourable results in rats by injecting glutathione or  $\beta$ -mercaptoethylamine after irradiation, provided that the region of the liver had been protected by a lead screen. Neither STRAUBE and PATT<sup>4</sup> nor various other investigators have confirmed this, and would appear as if Maisin and his collaborators had not mentioned one or more important conditions in their experiments.

HELLER<sup>8</sup> thinks it is possible to improve the condition of irradiated animals by stimulating the reticulo-endothelial system, especially by injecting choline. This is a new possibility, which can be studied quantitatively by the method which Heller devised, testing the phagocytosis of  $\text{Cr}^{32}\text{PO}_4$  by the reticulo-endothelial system. Another possible method is to use in the nutrition of the organism a metabolite the oxidation of which is increased by irradiation. In this way *Tetrahymena pyriformis* were restored with acetate<sup>10</sup>.

## REFERENCES

- <sup>1</sup> LATARJET, R., *Symp. Radiobiol.* (ed. J. J. Nickson), Wiley, New York, 1952
- <sup>2</sup> BLUM, H. F., ROBINSON, J. C., LOOS, G. M., *J. gen. Physiol.*, 1951, **35**, 323
- <sup>3</sup> MAISIN, J. H., LAMBERT, G., MANDART, M. and MAISIN, H., *Nature, Lond.*, 1953, **171**, 971
- <sup>4</sup> STRAUBE, R. L. and PATT, H. M., *Proc. Soc. exp. Biol. Med.*, 1953, **84**, 702
- <sup>5</sup> LAMARQUE, P., *Presse Méd.*, 1952, **60**, 1039
- <sup>6</sup> HOLTHUSEN, H., in G. F. Haenisch and H. Holthusen *Einführung in die Röntgenologie*, G. Thieme Verlag, Leipzig, 1933
- <sup>7</sup> HOLLAENDER, A. and STAPLETON, G. E., *Physiol. Rev.*, 1953, **33**, 77
- <sup>8</sup> HELLER, J. H., *Abstr. Commun., XIX intern. physiol. Congr., Montreal*, 1953, p. 450
- <sup>9</sup> LATARJET, R., *Acta Radiol.*, 1954, **41**, 84
- <sup>10</sup> EICHEL, H. J. and ROTH, J. S., *Biol. Bull.*, 1953, **105**, 373
- <sup>11</sup> STAPLETON, G. E., BILLEN, D. and HOLLAENDER, A., *J. cell. comp. Physiol.*, 1953, **41**, 345

## INTERVENTION OF THE PITUITARY AND ADRENAL GLANDS 'STRESS' IN RADIATION SICKNESS

### STRESS AND THE ADAPTATION SYNDROME

THE biochemical and physiological study of irradiated mammals is complicated by the complex of non-specific neuro-endocrine reactions which H. Selye has called the adaptation syndrome. Detailed discussions of this question are to be found in the works of SELYE<sup>1-2</sup> and extensive reviews in various textbooks, among them BACQ's<sup>3</sup>. The essence of this concept is that every strongly harmful agency (=stress\*) stimulates the hypothalamic nerve centres (either directly or through the mediation of adrenaline) and these control the activity of the anterior pituitary, the source of several hormones. The secretion of three hormones is inhibited: *prolactin*, which maintains milk secretion, the *gonadotrophic hormone* which activates the sex glands, and the *growth hormone*, recently named the *somatotropic hormone* (STH). On the other hand the secretion of the *adrenocorticotropic hormone* (ACTH) is increased. It is said colloquially that the synthesis of an excess of ACTH takes place at the expense of the other anterior pituitary hormones. As a result of this discharge of ACTH the cortex of the adrenal gland becomes overactive and discharges into the blood an abnormally large quantity of steroid hormones†, so-called because they are derived from the sterane nucleus. These corticoids act on the whole body, are eliminated in the urine, and are partly inactivated by conjugation.

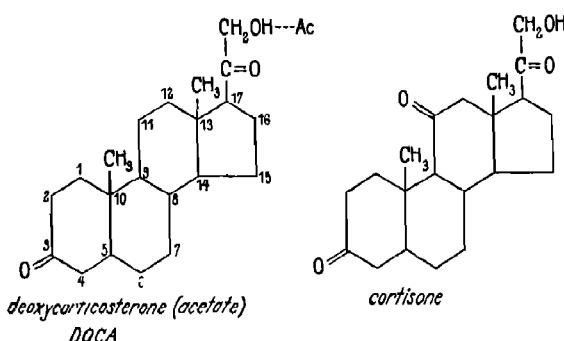
All the anterior pituitary hormones are proteins, and can therefore be estimated only by measuring their biological effects. The presence of ACTH has been demonstrated in the blood, and the estimation of prolactin and gonadotrophic hormone in the blood is current practice. If the adrenal glands have been removed, injections of ACTH have no effect, as this hormone acts only through the

\* Examples of stress: Trauma, haemorrhage, operation, intense cold, u.v. radiation, ionizing radiation; injection of chemical substances (formol, colchicine, salicylates); strong emotional excitement; infectious disease, intoxications.

† They are also called corticoids.

## INTERVENTION OF THE PITUITARY AND ADRENAL GLANDS

mediation of the adrenal cortex. More than thirty sterane derivatives have been isolated by chemists from extracts of this gland (obtained from oxen, sheep and whales), but this does not mean that all are physiologically active, or discharged into the blood or are true hormones\*. More refined methods of detection have recently been adopted (paper chromatography and electrophoresis), which make it possible to study the cortical hormones as they occur in the blood. Only two or three have been discovered up to the present and they seem to differ in different species of mammals. Two groups of steroids can be determined chemically in the urine: (i) those with a ketone group in position 17, and (ii) those with a second C=O in position 11. It appears that this characteristic position of the C=O groups is related to important physiological effects. The 17-ketosteroids act mainly on the distribution of the Na and K ions, and are therefore called mineralo-corticoids. Deoxycorticosterone is of this type. It has been synthesized, and is used in the form of an ester, DOCA, or deoxycorticosterone acetate.



*Figure 1. Deoxycorticosterone acetate (DOCA) and cortisone*

The 11-oxysteroids, of which the only one used in practice is cortisone, act on the carbohydrate, protein and lipid metabolism; they are the gluco-corticoids. In a normal mammal, endocrine regulation depends not only on the total quantity of steroids secreted, but also on the proportion of gluco- and mineralo-corticoids. An excess of mineralo-corticoids leads to sodium retention, arterial lesions and impairment of the heart and kidneys; an excess of cortisone disturbs the physiology of the connective tissue, promotes the spread of infections, and causes involution of the lymphoid tissues, hyperglycaemia and even diabetes.

---

\* Many are precursors, others are artefacts produced by extraction, as chemical extraction procedures are necessarily severe.

## STRESS AND THE ADAPTATION SYNDROME

If an animal is subjected to intense, continuous stress (*e.g.* cold), the pituitary-adrenal reaction takes place in three phases: (i) *alarm*, which lasts several days (shock and counter-shock), during which the adrenal gland hypertrophies and gradually increases its secretion; (ii) *resistance*, during which the animal shows increased resistance to cold, due to the adrenal reaction, which may last for weeks; (iii) *exhaustion*, which leads sooner or later to death, if the stress is too great or the animal is not in perfect physical condition. The adrenal gland is exhausted and can no longer supply the body needs. Death occurs in the state of weakness characteristic of Addison's disease, or adrenal insufficiency.

During the counter-shock and early resistance phase, the mammal is slightly more resistant to stresses other than that which produced the reaction, in other words there is *cross-resistance*. For instance, an animal adapted to cold will be a little less sensitive to injury or to u.v. light.

The ability to react, or reserve power, of a mammal is conditioned to a great extent by its state of nutrition. On a diet rich in protein the adaptation syndrome is very distinct; on a poor, mainly carbohydrate diet, the reactions to stress are much weakened. An excess of sodium in the diet favours the occurrence of lesions due to an excess of mineralo-corticoids; on the other hand an excess of sodium is very helpful in combating adrenal insufficiency.

## TECHNIQUES USED IN STUDYING THE REACTION TO STRESS

The problem arises of following the various phases of reaction to stress in animal experiments. The determination of ACTH in the blood is too difficult, and the adrenal response is not necessarily proportional to the amount of ACTH in the blood stream. It is therefore better to approach the problem in the adrenal glands. The ideal would be to trace the 17-ketosteroids and 11-oxysteroids in the blood hour by hour, but neither our chemical nor our biological methods make this possible. The 17-ketosteroids and 11-oxysteroids can be estimated separately in the 12-hour or 24-hour urine, and their excretion can be followed for weeks<sup>27, 28</sup>, but contamination by sex hormones, which are also steroids, and by their metabolites, must not be forgotten.

The adrenal gland should always be studied simultaneously by histological and chemical methods. The corticosteroids in the normal adrenal cortex are fat-soluble. They are stored in solution in the lipids which give the gland its characteristic yellowish colour, and when excreted they leave the gland with their solvent.

## INTERVENTION OF THE PITUITARY AND ADRENAL GLANDS

Therefore a gland with a good supply of fats is neither exhausted nor over-active. When the fats completely disappear and there is hypertrophy, anatomists deduce increased activity, the reserves being exhausted, and the synthesized steroids discharged immediately into the blood. It is difficult to decide by this anatomical method alone when exhaustion begins. It is in any case dangerous to assume hyper- or hyposecretion merely from the histological appearance of a gland.

In the gland, the synthesis of corticosteroids begins from cholesterol, and consumes ascorbic acid. Very active synthesis is therefore associated with a simultaneous decrease in the cholesterol and ascorbic acid content of the gland. During normal activity the cholesterol and ascorbic acid brought by the blood (or, in some animals, synthesized in the organ) exactly balance the quantities used during the constant synthesis of corticoids. This decrease in concentration can only be used to detect great increases in secretion, and it can only be used in large series of small rodents (mice, rats) as an animal must be killed for each estimation.

There are several fairly specific reactions for cortisone which indicate the presence of gluco-corticoids in the blood: (a) a fall of at least 80 per cent in the number of circulating eosinophiles, (b) the excretion of uric acid in the urine, shown by a great increase in the uric acid/creatinine ratio, (c) a fall in the number of circulating lymphocytes; this is Thorn's test, widely used in man, but also applicable to animals.

There is no such sensitive and specific test for mineralo-corticoids; the most valid tests for hypersecretion of 17-ketosteroids are those which reveal sodium retention and increased potassium elimination.

Many authors make the serious mistake of accepting two, or sometimes even one anatomical, histochemical or chemical sign as evidence of a pituitary-adrenal reaction. For example, the ascorbic acid content of the adrenal may fall to one-third of its value after an injection of cysteamine in the rat without any change in the cholesterol content or in the number of circulating eosinophiles<sup>19</sup>, or in the excretion of corticoids, or in the uric acid/creatinine ratio<sup>20</sup>. Yet in many papers, a fall in the ascorbic acid content is the only foundation for the assertion that stress is present.

It is also incorrect to believe that all the ascorbic acid which disappears from the adrenals is used in the synthesis of corticoids. This is not known. The liver contains a large quantity of ascorbic acid and stress (cold or x-rays) causes increased excretion of dehydroascorbic and diketogulonic acids, two forms of oxidized ascorbic acid<sup>21</sup>.

## DO IONIZING RADIATIONS ACT AS STRESS?

### DO IONIZING RADIATIONS ACT AS STRESS?

It is difficult to interpret all the aspects of radiation sickness in the light of the adaptation syndrome, but it would be wrong to deny the interest of Selye's concept, which collates a very large number of phenomena in vertebrate radiobiology, of which the most important are:

(1) *The production of radioresistance by sublethal irradiation* has been found in mice by all who have looked for it, whether with  $\beta$ -rays (RAPER<sup>4</sup>) or x-rays (DOWDY<sup>24</sup>, CRONKITE *et al.*<sup>5</sup>, BETZ<sup>6</sup>) and whether one or more preliminary irradiations were given. For a perfect demonstration the sublethal irradiation must not kill any animal, otherwise it selects the most radioresistant animals. By using a single sublethal irradiation, it is possible to study the course of this radioresistance. In 57 mice, 500 r produces a mortality of 10 to 15 per cent; if 700 r (a 100 per cent fatal dose) is given 7, 10, 15 or 33 days after the 500 r dose, the survivals recorded are 20, 26, 36 and 0 per cent respectively, that is to say, the radioresistance is greatest on about the 15th day, and disappears completely by the 33rd day<sup>6</sup>. These intervals are compatible with Selye's concept. Quantitatively, the induced radioresistance is slight.

(2) *The existence of cross resistance*. If a dose or irradiation fatal to all control mice is given after stimulation of the adrenal cortex by stress or by an adequate chemical stimulus, some of the animals survive: 15 per cent if cold is the stimulus (1 hour in the refrigerator every day for 7 to 30 days), 25 to 50 per cent after sodium salicylate (400 to 600 mg/kg daily for 12 days), 22 per cent after ACTH, 20 to 40 per cent after DOCA, and none after cortisone. If the cold or salicylate or ACTH or cortical hormones are used after irradiation, death occurs more quickly than in controls (BETZ<sup>6</sup>; see also<sup>37</sup>).

(3) Irradiation of the pituitary gland only, using a beam of energetic electrons from a Van de Graaff generator, increases the ACTH content of the blood within an hour, and the increase lasts for at least 24 hours (MATEYKO *et al.*<sup>18</sup>).

(4) Adrenalectomized rats seem to be more sensitive than normal rats to x-rays (EDELMAN<sup>7</sup>, HALPERN *et al.*<sup>8</sup>). CRONKITE and CHAPMAN<sup>16</sup> have confirmed this in mice. The conclusion reached by STRAUBE *et al.*<sup>17</sup>, that 'the radiosensitivity of intact or adrenalectomized mice, with or without exogenous adrenal cortical steroids, is similar' is therefore in part erroneous. Whereas administration of a total cortical extract of DOCA prolongs the life of irradiated rats, whether adrenalectomized or not, it is claimed that cortisone

## INTERVENTION OF THE PITUITARY AND ADRENAL GLANDS

has no effect or may even *increase* the mortality of irradiated rodents during a period beginning on the fifth day after irradiation, that is, at the time when infections show themselves<sup>8-13</sup>. Cortisone is clearly contra-indicated in radiation sickness. LANGENDORFF *et al.*<sup>40</sup> have clearly demonstrated a small but definite protective effect with mice if DOCA or total extracts from the adrenal are injected or adrenals are implanted. This protective action is only observed if doses are given which do not kill all the animals (500 r) and not if the minimum 100 per cent lethal dose (650 r) of x-rays is given.

In rabbits a moderate irradiation (200 r to 480 r) suppresses the cutaneous reaction to tuberculin either partially or completely, even if the skin at the site of the test is protected from the rays by a lead screen. LENNOX and his collaborators<sup>30</sup>, who observed this fact, believe it to be due to overproduction of cortisone.

Protection of the adrenal glands by a lead screen reduces the mortality of rats after doses of 700 or 800 r applied to the whole body<sup>14</sup>, but it must be admitted that, here too, the protection is slight, much less than that obtained with chemical protectors (see Chapter 14). EDELMAN<sup>14</sup> supposes that an adrenal gland which has not been irradiated keeps its ability to respond to an excess of ACTH secreted by the anterior pituitary by discharging an increased quantity of corticoids.

At this point difficulties are encountered, and it begins to appear that whole-body irradiation is a very special form of stress.

## DIFFICULTIES IN FACTS AND INTERPRETATIONS

(1) One of the characteristic signs of the alarm reaction is increased protein catabolism, which leads to an excess of urea in the urine, or, after removal of the kidneys, to a very rapid rise in blood urea. This occurs even after hypophysectomy, and does not depend on a reaction of the pituitary. However, between 3½ and 48 hours after irradiation ureaemia develops in the same way in nephrectomized, irradiated rats and in nephrectomized controls, whereas injections of adrenaline or formol produce a sudden rise in the blood urea<sup>34</sup>.

(2) CRONKITE<sup>13</sup> says 'BOND *et al.*<sup>15</sup> have conclusively demonstrated that radiation, when used *locally* in doses sufficient to produce destruction of tissue, will produce the alarm reaction in the manner described by Selye'. Not everything that is observed after local irradiation is to be interpreted as necessarily due to this reaction. LEBLOND and SEGAL<sup>31</sup> have, however, shown that some of the reactions at a distance are produced by way of the adrenals. For instance, the atrophy of the rat thymus which follows irradiation of the

#### DIFFICULTIES IN FACTS AND INTERPRETATIONS

abdomen (2000 to 3700 r) does not occur in animals adrenalectomized 2 to 5 days before irradiation; similarly the atrophy of the spleen which follows irradiation of the head and neck (2800 r) does not occur after adrenalectomy.

It must not be forgotten that during total irradiation of a mammal the glands which will have to react to the stimulation of the hypothalamus, *i.e.* the pituitary and adrenal, are themselves irradiated. They may be directly affected so that the response of the pituitary to the nervous stimulus and of the adrenals to ACTH may be different. Nobody has yet made a quantitative study of the response of *locally irradiated* adrenals to ACTH in a recently hypophysectomized rat. If the anatomical appearance of an organ (*e.g.* lipoid accumulation in the liver) is to be taken as a test for the action of the corticosteroids, it should be possible to allow for the fact that this liver has been irradiated, but it is impossible to assess this variation quantitatively.

(3) It is dangerous to argue by analogy. The fact that in mammals, cortisone, DOCA or ACTH produce tissue changes and some of the symptoms seen after irradiation, does not mean that in irradiated animals these changes are due to the neuro-hypophyso-adrenal reaction. It is unfortunate that the undeniable specific effects of ionizing radiations are often identical with those of one or other of the cortical hormones, and cannot be separated from them. The following is a typical example, and others could be given. Isolated lymph glands, in tissue cultures, and therefore free from all nervous and hormonal influences, are highly radiosensitive. TROWELL<sup>29</sup> using this material, carried out some excellent quantitative studies. The same tissue, cultivated in the same conditions reacts to very low concentrations (0.1 µg/ml) of cortisone; a concentration of  $3 \times 10^{-5} M$  of cortisone kills 45 per cent of the lymphocytes in two days. It is impossible to distinguish between lymphocytes damaged by cortisone and lymphocytes killed by x-rays; both show the same pyknotic degeneration. To avoid the intervention of the corticoids it is necessary to use either isolated organs or adrenalectomized mammals. Another difficulty arises here. The rat is about the only mammal which tolerates adrenalectomy well; the others become very frail, and many are lost even if they are given regular injections of cortical adrenal hormones and sodium chloride. The reactions of adrenalectomized mammals to ionizing radiations become irregular, and a quantitative study is almost impossible. The rat tolerates loss of the adrenal glands better than other animals because it often has accessory adrenals, that is, islets of cortical tissue which become hypertrophic after removal of the true adrenals. For this reason

## INTERVENTION OF THE PITUITARY AND ADRENAL GLANDS

even adrenalectomized rats can only be used validly during the first fortnight after the operation. The hypophysectomized rat can be used for a longer period, but it suffers from multiple endocrine deficiencies which make it very different from the normal animal. Comparison of the reactions of organs *in vivo* and *in vitro* is certainly an excellent method. The techniques of Wolff and of Trowell, which make cultures of whole mammalian and avian organs possible, fortunately seem to be easier than was supposed a few years ago, and they are capable of being greatly developed.

The difficulties raised by this similarity of the effects of the corticoids and of x-rays are implicitly recognized by Ellinger, who, ever since 1941, has been emphasizing the importance of cortical adrenal *insufficiency* in radiation sickness. The facts he stresses are metabolic phenomena which might be caused by cortical insufficiency, and are difficult to interpret by taking into account the specific action of the radiations alone. These phenomena are 'loss of chlorides, disturbances of water metabolism, fall in blood cholesterol levels with increased fat deposits in the liver, a biphasic response of the hydrogen-ion concentration in blood and tissues and of the blood sugar'\*. The disturbances in ion distribution are genuine. In rats, after a dose of 900 r applied to the whole body, the urinary excretion of potassium is increased, and that of sodium is decreased, compensating for increased elimination in the faeces. The radiosensitive tissues show first a fall and then a considerable rise in sodium content after irradiation<sup>26</sup>. These changes in rats are in a direction suggesting cortical overactivity. In dogs or in man suffering from severe radiation sickness such studies would be senseless because the vomiting makes the loss of water, chloride and bases very variable. The dehydration and loss of nutrition by themselves explain the electrolytic changes observed in dogs after 450 r<sup>36</sup>.

(4) Many histologists are struck by signs of adrenal hyperactivity, but if their findings (or rather their interpretations) are compared with the changes in the cholesterol and ascorbic acid content of the adrenals there is a striking absence of agreement. For example, in the male rat (the phenomenon is less marked in the female) the ascorbic acid content of the adrenals falls 1 hour after irradiation (625 r) from 4.44 to 2.28 mg/g. At 3 hours it has risen to 3.01 mg/g, at 6 hours to 3.96 mg/g, at 12 hours to 4.14 mg/g and at 24 hours to 4.93 mg/g; 96 hours after irradiation the ascorbic acid content is normal (4.44 mg/g fresh tissue) although the weight of the gland has doubled<sup>22</sup>. The cholesterol content of the adrenals of the rat after

---

\* Recent work shows that increased fat deposits in the liver are caused by an excess of cortisone.

### DIFFICULTIES IN FACTS AND INTERPRETATIONS

650 r varies in approximately the same way<sup>23</sup>: controls, 30.4 mg/g; 6 hours after irradiation, 14.2 mg/g; 2 days after irradiation, 24.8 mg/g; 4 days after irradiation, 34.4 mg/g. The fall is probably more abrupt than these figures show, for after 900 r the cholesterol content falls from 26 to 14.4 mg/g in 3 hours. Unfortunately Patt and his collaborators did not kill any animals 1 hour after irradiation. These figures suggest a rapid utilization of ascorbic acid and cholesterol during the first hour after irradiation, with a return to the normal level in about 24 hours.

Recent experiments at Liège<sup>39</sup> confirm the findings of WERKER *et al.*<sup>22</sup> and of PATT *et al.*<sup>23</sup> concerning the immediate effect on the suprarenal and restoration after 24 hours (see *Table I*). A second

*Table I. Effect of Irradiation on the Suprarenals of Rats and the Protective Action by Cysteamine<sup>39</sup>*

	Hours after irradia- tion	No. of rats	Wt of supra- renal mg/100 g body wt	Ascorbic acid in suprarenals mg/100 g	Ascorbic acid in liver mg/100 g	Cholesterol in suprarenals mg/100 g
<i>Controls</i>		116	10.5(±1.2)	450(±93)	31.2(±6)	4.57(±1.5)
<i>Irradiated with 800 r</i>	1	22	10.3(±1.2)	275(±80)	34.5(±7.2)	2.98(±0.6)
	2	10	10.6(±1.7)	252(±23)	33.7(±4.3)	2.55(±0.7)
	3	22	11.4(±1.3)	288(±39)	33.5(±5.4)	2.81(±1.1)
	5	10	11.2(±0.5)	387(±32)	30.5(±2.7)	3.96(±1.7)
	24	12	11.2(±0.3)	457(±70)	35.1(±3.8)	3.9(±1.5)
	48	6	14.6(±0.4)	407(±47)	35.6(±2.7)	3.29(±0.8)
	72	12	20(±1.3)	242(±28)	41(±1.9)	1.31(±0.5)
	96	6	24(±0.6)	284(±59)	36.7(±5.8)	0.92(±0.6)
<i>Cysteamine 10 mg/100 g before ir- radiation with 800 r</i>	1	17	10.3(±0.4)	<i>No data because cysteamine affects ascorbic acid content of tissues during this period.</i>		3.17(±0.8)
	2	10	10.3(±1.4)			2.45(±1.5)
	3	12	11.5(±0.9)			2.38(±0.8)
	5	10	10.4(±1)			3.58(±0.8)
	24	8	11.2(±0.8)	448(±29)	33.9(±2.6)	4.05(±0.7)
	48	6	10.5(±0.8)	412(±71)	33.7(±1.8)	3.92(±0.6)
	72	12	12(±1.5)	483(±60)	33.5(±3.8)	4(±1)
	96	6	12.3(±1.7)	453(±46)	30.3(±1.8)	4.21(±1.4)

drop, however, occurs after the third or fourth day which was probably not detected by the other workers because they used a smaller dose of radiation. If the protective agent cysteamine (see Chapter 14) is injected immediately before irradiation, the condition of the suprarenals at 24 hours is the same as that of the irradiated rats and the same drop in cholesterol content is observed after 2 hours but there is no second drop on the third and fourth day; these animals would have survived. Similarly, the slight increase in liver ascorbic

#### INTERVENTION OF THE PITUITARY AND ADRENAL GLANDS

acid most marked 72 hours after irradiation is not seen if the rat has received an injection of cysteamine. The weight of the suprarenal increases after 48 to 96 hours in irradiated rats (from 10.5 mg/100 g to 24) but not in cysteamine protected animals.

In the mouse (after 700 r) on the other hand, histologists find a progressive diminution in the fat content of the adrenal cortex, which is most marked on the sixth to seventh day<sup>6</sup>. There is no temporal agreement between the anatomical and the chemical findings and a similar effect has also been observed by MATEJKO *et al.*<sup>18</sup>, who found in rats that the corticotrophic hormone of the pituitary increases during the first hour after irradiation (1000 r to the whole body, with the head protected); it begins to fall after about 6 hours and reaches a subnormal level after 24 hours. There are two possibilities; either this disagreement is due to the difference in the animal material and in the technique of irradiation; or there is, in fact, no agreement. In the latter case it must be determined whether the error is in the interpretation of the chemical estimations or in that of the histological appearances. This brief analysis shows the great importance of a twofold study, anatomical and biochemical, of the irradiated animals. If a prolonged study of this kind is not undertaken, the anatomists will continue to prefer their own interpretation, and the biochemists will persist in not paying due attention to anatomical observations.

(5) CRONKITE<sup>13</sup> and KOHN<sup>38</sup> emphasize at some length that it is impossible to form a clear picture of the participation of the pituitary and adrenals in radiation sickness.

One of the major disadvantages of Selye's concept as applied to radiation sickness is its excessive versatility. If some fact is not explained by hypersecretion of cortisone, its antagonist, corticosterone, is brought in, or some argument is advanced which tends to prove that the gland becomes exhausted and that there is a temporary insufficiency. When it is remembered that certain accurate experiments give undeniable evidence that other pituitary hormones intervene\*, that the pituitary is inhibited by an excess of sexual, thyroid or cortical hormones in the circulation, we are confronted by

---

\* SELYE, SAGEDO and PROCOPIO<sup>33</sup> show that daily administration of pituitary growth or somatotrophic hormone (STH) does not decrease the death rate, but ensures normal growth in rats subjected to a sublethal dose, and prevents the involution of the thymus, spleen and liver. According to BETZ<sup>41</sup> STH injected to rats (5 international units) daily after irradiation (800 r) increases survival only in adrenalectomized animals. GORDON *et al.*<sup>42</sup> have obtained negative results in mice. In the guinea-pig and the rat LACCASAGNE and TUCHMANN-DUPLESSIS<sup>43</sup> have observed a slight decrease of radiosensitivity in a narrow range of x-ray doses, near the threshold lethal dose; according to these authors, STH is active in the guinea-pig only if given before irradiation.

#### DIFFICULTIES IN FACTS AND INTERPRETATIONS

a fantastic dialectical labyrinth, into which it is advisable not to enter without great caution. It is high time to abandon purely anatomical or indirect chemical methods and to attack this difficult problem directly, identifying and estimating the cortical adrenal hormone in the adrenal vein or, at least, in the urine. Whatever the appearance and contents of the gland, what is important to the body at the moment of observation is the hormone secretion and the concentration of these molecules in the blood.

As far as is known, studies of this kind have been carried out only in dogs. LAWRENCE<sup>25</sup> has observed a constant increase in the excretion of 17-ketosteroids in the urine, in male and female dogs, at various times between the fifth and the twelfth day, most frequently about the fifth day. It is probable that this is due to the reaction to the shock caused by haemorrhage and infection rather than to the effect of irradiation itself. This observation has not been confirmed by DUFFY<sup>35</sup> who found no significant change in the urinary excretion of 17-ketosteroids in 8 dogs after a minimal lethal dose of 450 r. The dog does not seem to be a suitable animal for this kind of study.

To conclude, the difficulties encountered in interpreting the facts of radiobiology in vertebrates only increase the value of observations on living material which is not subject to neurohumoral regulation. If a fact observed in a mouse is observed in micro-organisms, in pollen and in growing roots, it is certain that it is a fact of *general radiobiology*. Mammalian reactions show peculiarities which are important because they also concern man, and in this atomic age it is difficult not to think of oneself sometimes, but this is a matter of *special radiobiology*. Mammals have special nervous and humoral regulating mechanisms; they have certain substances which other organisms (erroneously regarded as 'inferior') do not possess; they also have special needs, being unable to synthesize many molecules which unicellular organisms produce easily.

Further chapters will be devoted to the special radiobiology of mammals and of man, but they will be summaries, the main object of which will be to show that the reactions of these animals can best be understood in the light of general radiobiology, which we have tried to define in the preceding chapters.

#### REFERENCES

- <sup>1</sup> SELYE, H., 'The physiology and pathology of exposure to stress', *Acta Inc.*, Montreal, 1950; Supplements 1951 and 1952
- <sup>2</sup> SELYE, H., *Brit. med. J.*, 1950, **2**, 1383
- <sup>3</sup> BACQ, Z. M., 'Principes de physiopathologie et thérapeutique générales', *Sciences et Lettres*, Liège; Masson, Paris, 1949

INTERVENTION OF THE PITUITARY AND ADRENAL GLANDS

- <sup>4</sup> RAPER, J. R., *Radiology*, 1947, **49**, 314
- <sup>5</sup> CRONKITE, E. P., SIPE, C. R., ELTZHOLTZ, D. C., CHAPMAN, W. H. and CHAMBERS, F. W. Jr., *Proc. Soc. exp. Biol. Med.*, 1949, **70**, 125
- <sup>6</sup> BETZ, H., *Bruxelles médical*, 1953, **33**, 121  
BETZ, H., *C.R. Soc. Biol.*, 1950, **144**, 1439
- <sup>7</sup> EDELMANN, A., *Amer. J. Physiol.*, 1951, **167**, 345
- <sup>8</sup> HALPERN, B. N., CUENDET, A. and MAY, J. P., *J. suisse Méd.*, 1952, **82**, 1020
- <sup>9</sup> GRAHAM, J. B., GRAHAM, R. M. and GRAFFEO, A. J., *Endocrinology*, 1950, **46**, 434
- <sup>10</sup> SMITH, W. W., SMITH, F. and THOMPSON, E. C., *Proc. Soc. exp. Biol. Med.*, 1950, **73**, 529
- <sup>11</sup> SYVERTON, J. T., WERDER, A. A., FRIEDMAN, J., ROTH, F. J., GRAHAM, A. B. and MIRA, O. J., *ibid.*, 1952, **80**, 123
- <sup>12</sup> ELLINGER, F., *ibid.*, 1952, **80**, 214
- <sup>13</sup> CRONKITE, E. P., *Atomic Medicine*, Williams and Wilkins, Baltimore, 1953, pp. 179-218
- <sup>14</sup> EDELMANN, A., *Amer. J. Physiol.*, 1951, **165**, 57
- <sup>15</sup> BOND, V. P. et al., *Amer. J. Physiol.*, 1950, **161**, 323
- <sup>16</sup> CRONKITE, E. P. and CHAPMAN, W. H., *Fed. Proc.*, 1950, **9**, 329
- <sup>17</sup> STRAUBE, R. L., PATT, H. M., TYREE, E. B. and SMITH, D. E., *Proc. Soc. exp. Biol. Med.*, 1949, **71**, 539
- <sup>18</sup> MATEYKO, G. M., EDELMANN, A., CHARIPPER, H. A. and GORDON, A. S., *Amer. J. Physiol.*, 1951, **167**, 190
- <sup>19</sup> VAN CAUWENBERGE, H., ROSKAM, J., HEUSGHEM, C., FISCHER, P., DELTOUR, G. and BACQ, Z. M., *Arch. int. Physiol.*, 1953, **61**, 124
- <sup>20</sup> BACQ, Z. M., BERNARD, J., RAMIOL, H. and DELTOUR, G., *Bull. Acad. roy. Méd. Belg.* 1952, 6th series **17**, 460
- <sup>21</sup> MONIER, M. M. and WEISS, R. J., *Proc. Soc. exp. Biol. Med.*, 1952, **81**, 59
- <sup>22</sup> WERKER, B. C., PENCHARZ, R. and THOMAS S. F., *ibid.*, 1952, **79**, 183
- <sup>23</sup> PATT, H. M., SWIFT, M. N., TYREE, E. B. and JOHN E. S., *Amer. J. Physiol.*, 1947, **150**, 480
- <sup>24</sup> DOWDY, A. H., *Univ. Rochester Atom. Energy Proj., Quart. Rep.*, July 1-30, Sept. 30, 1947, p. 12
- <sup>25</sup> LAWRENCE, G. H., *Endocrinology*, 1949, **45**, 383
- <sup>26</sup> BOWERS J. L. and SCOTT, K. G., *Proc. Soc. exp. Biol. Med.*, 1951, **78**, 645-648
- <sup>27</sup> HEUSGHEM, G., 'Métabolisme et analyse des hormones stéroïdes', *Actual. biochim.*, No. 14, Desoer, Liège, 1950
- <sup>28</sup> LIEBERMAN, S. and TEICH, S., *Pharmacol. Rev.*, 1953, **5**, 285
- <sup>29</sup> TROWELL, O. A., *J. Physiol.*, 1953, **119**, 274
- <sup>30</sup> LENNOX, B., DEMPSTER, W. J., BOAG, J. W., *Brit. J. exp. Path.*, 1952, **33**, 380
- <sup>31</sup> LEBLOND, C. P. and SEGAL, G., *Amer. J. Roentgenol.*, 1949, **62**, 547
- <sup>32</sup> ELLINGER, F., *Proc. Soc. exp. Biol. Med.*, 1952, **81**, 486
- <sup>33</sup> SELYE, H., SALGADO, E. and PROCPIO, J., *Acta Endocrinol.*, 1952, **9**, 337
- <sup>34</sup> THOMPSON, J. F. and MIKUTA, E. T., *Argonne nat. Lab., Quart. Rep.*, 1953, ANL-4948, p. 99

#### REFERENCES

- <sup>35</sup> DUFFY, B. J. Jr., *Univ. Rochester Atom. Energy Proj.*, 1951, UR-164, p. 13
- <sup>36</sup> MASON, W. B., COULTER, M. P. and FURTH, F. W., *Univ. Rochester Atom. Energy Proj.*, 1951, UR-189, p. 16
- <sup>37</sup> *Univ. Rochester Atom. Energy Proj.*, 15 Oct. 1948, UR-45, p. 35
- <sup>38</sup> KOHN, H. I., *Amer. J. Physiol.*, 1951, **165**, 43
- <sup>39</sup> FISCHER, P., BEAUMARIAGE, M. L. and BACQ, Z. M., *Bull. Acad. Med. Belg.*, 1954, in press
- <sup>40</sup> LANGENDORFF, H., KOCH, R. and SAUER, H., *Strahlentherapie*, 1954, **93**, 37, 44, 381
- <sup>41</sup> BETZ, E. H., *Symposium de Radiobiologie*, Liège, 1954, Butterworths, London, 1955
- <sup>42</sup> GORDON, L. E., MILLER, C. P. and HAHN, N. J., *Proc. Soc. exp. Biol. Med.*, 1953, **83**, 85
- <sup>43</sup> LACASSAGNE, A. and TUCHMANN-DUPLESSIS, H., *C.R. Acad. Sci.*, 1953, **236**, 540
- <sup>44</sup> BRAYER, F. T., GLASSER, S. R. and DUFFY, B. J. Jr., *Science*, **120**, 112
- <sup>45</sup> ABDERHALDEN, R., *Strahlentherapie*, 1940, **68**, 17

## PHYSIOPATHOLOGY AND TREATMENT OF RADIATION SICKNESS IN MAMMALS

MANY hundreds of papers have been devoted to this subject because it is directly related to the interpretation of the signs observed in man. There can be no question of reviewing them here—many are, in any case, of little general interest. A general sketch will be sufficient, since there are recent reviews by HALEY<sup>31</sup>, PATT<sup>32</sup> and LOUTIT and MAYCOCK<sup>33</sup>. The only subject which has been discussed at greater length is the participation of the pituitary-adrenal endocrine system in the preceding chapter.

### EXTREME IRRADIATION

If a mammal is left under a powerful x-ray tube or a  $^{60}\text{Co}$   $\gamma$ -ray source giving 15,000 r per hour, it dies in 1½ to 3 hours, the cause apparently being the very greatly increased potassium content of the blood. The cells take up sodium in exchange for the potassium they lose; in other words the action of the sodium pump is blocked (LOUTIT<sup>16</sup>). Very recent work by KOCH<sup>14</sup> shows that cholinesterase is one of the components of the enzymatic system which ensures the active expulsion of the sodium ion from the interior of the cells, and the absorption of this sodium by the gills and anal papillae of certain fresh-water invertebrates. The inhibition of a cholinesterase after irradiation has been observed *in vivo* (BURN, KORDIK and MOLE<sup>6</sup>), and ionizing radiations may therefore be useful in studying the mechanism of the sodium pump.

The dog is the animal in which the reactions to ionizing radiations are most akin to those found in man. Rodents are preferred for studies on protection and on biochemical effects, because they lend themselves better to serial experiments. It is sometimes dangerous to transfer anatomical and clinical observations from the mouse or rat to man. For instance, the spleen of the adult mouse produces erythrocytes, whereas in man only the foetal spleen is erythropoietic. The irradiated mouse does not die from anaemia, which is one of the cardinal symptoms of radiation sickness in man. In small rodents the majority (60 per cent) of the leucocytes are lymphocytes,

## EXTREME IRRADIATION

whereas in man the granulocytes produced in the bone-marrow are by far the most numerous (55 to 80 per cent).

The dose of x-rays fatal to 100 per cent of dogs is 450 r; many workers choose a slightly higher dosage (450 to 500 r) when they wish to study the earliest phases of radiation sickness.

## THE FIRST STAGE OF RADIATION SICKNESS

During the first half hour or the first few hours after moderate irradiation, or immediately after intense irradiation, there is a fall in blood pressure, apparently as a result of depression of the central nervous system and of liberation of histamine\*. All the signs of shock appear: after 3 to 4 days *the permeability of the capillary endothelium is increased*, to the extent of allowing the lymph to be invaded by red corpuscles<sup>22</sup>, the concentration of the blood is increased by vomiting, diarrhoea etc.; there is mass destruction of the red corpuscles which have invaded the lymph. The increased permeability of the capillaries persists until death; at autopsy the thoracic duct is often found to be as red as a vein.

This shock should be treated as any other, by careful attention to the electrolyte balance, the secretory function of the kidneys, the blood pressure and the concentration of the blood. Plasma substitutes are indicated at this stage.

The animal eats and drinks little, and loses weight. Involvement of the lymphatic system can be demonstrated anatomically within an hour or two. The lymphocytes disappear from the blood stream, firstly because they are not replaced by young cells, as these are not being formed; secondly because the life of the lymphocytes is short, and thirdly because there is rapid destruction of the irradiated lymphocytes in the blood. As early as 3 hours after a dose of 400 r, 7 per cent of the circulating lymphocytes of rats are pyknotic. These cells are removed from the blood stream, especially by the lungs, where they can be found in the alveolar capillaries<sup>20</sup>.

During the first 2 or 3 hours after irradiation hypergranulocytosis appears, due mainly to an increase in the circulating polynuclear neutrophiles of obscure origin. In the rat this leucocytosis ends in the twelfth hour. Leucopenia develops gradually, and reaches its maximum in 2 to 8 days. If the animal dies it will have very few

\* Ellinger has strongly emphasized this. W. Feldberg's classic investigations have shown that any physical stimulus of sufficient strength (e.g. exposure to x-rays) liberates histamine in the isolated guinea-pig lung. There is an increase in the blood histamine of irradiated rats<sup>21</sup>. Resorption of infected substances is decreased considerably by irradiation<sup>25</sup> and this may be due to a temporary inhibition in the secretion of ACTH<sup>26</sup>. Antihistamines increase the lethality of irradiation of mammals<sup>27, 28</sup> and are of doubtful use in radiation sickness.

PHYSIOPATHOLOGY AND RADIATION SICKNESS IN MAMMALS

white cells in the blood at the time of death. On the other hand, if it is to survive, regeneration of the leucocytes will begin to show after 8 to 10 days. In irradiated dogs there is close parallelism between

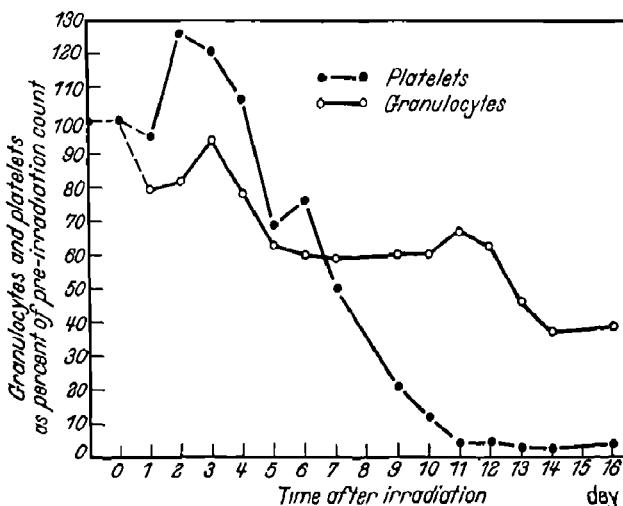


Figure 1. Mean granulocyte and platelet count on 10 dogs exposed to atomic bomb  $\gamma$ -radiation that produced 10 per cent mortality<sup>2</sup>

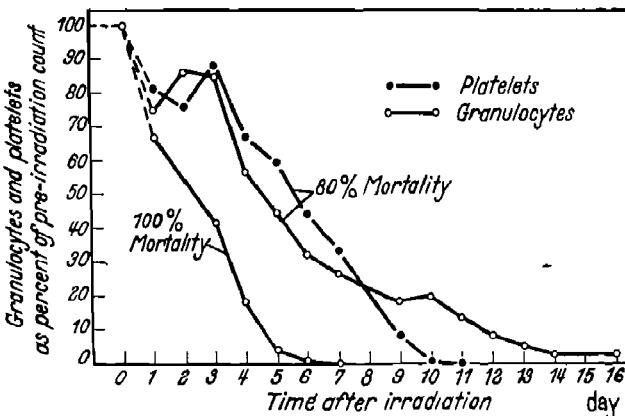


Figure 2. Mean granulocyte and platelet counts in 2 groups of 10 dogs receiving atomic bomb  $\gamma$ -radiation that produced 80 and 100 per cent mortality. Platelet response in 80 and 100 per cent groups were identical<sup>2</sup>

the rate at which leucopenia develops and the dose administered, in other words, between the leucopenia and the death rate (*Figures 1* and *2*). Regeneration of the leucocytes occurs later in dogs than in rats or mice.

#### THE FIRST STAGE OF RADIATION SICKNESS

Certain authors incorrectly maintain that there is a parallelism between the leucocyte count, resistance to infection and survival. *Figure 15*, p. 317 shows that mice irradiated with 750 r all die with extreme leucopenia, but it also shows that mice of the same strain, irradiated in the same way after an injection of cysteamine show the same leucopenia, but 9 out of 10 survive longer than 30 days. In these protected mice, regeneration of the leucocytes begins after the controls are dead. Considerable and sometimes lasting leucopenia has been produced in white men and negroes by injections of nitrogen mustard without their showing any kind of infection<sup>3,4</sup>. No form of treatment\* can prevent the rapid destruction of the circulating leucocytes or accelerate their regeneration. Even crossed-circulation established for 2 hours between a normal dog and one irradiated with 450 r 6 to 10 days earlier does not raise the number of leucocytes in the irradiated dog for more than 20 to 48 hours. Leucocytes supplied to an irradiated dog by mass transfusion do not remain long in the blood stream.

#### THE SECOND STAGE OF RADIATION SICKNESS

The second period is the period of haemorrhages, incipient anaemia, infection and malnutrition. *Haemorrhages* occur in nearly all irradiated dogs (450 r) and human beings; they vary in severity, in location, in their clinical course and in their response to treatment, and they are probably not the most frequent cause of death. In dogs they are most often found in the pericardium—not in the myocardium—and, in descending order of frequency, in the gastrointestinal tract, lungs, pleura and mediastinum, pharynx, skin, bladder and kidneys<sup>1</sup>. MOLE<sup>17</sup> emphasizes the many variations in the haemorrhages observed in different species of mammals.

Vascular fragility is one of the causes of these haemorrhages. The second cause is without doubt the disappearance of the blood platelets, even after non-lethal doses of radiation (compare *Figure 1*). A lethal dose (450 r) reduces the average number of circulating platelets in dogs from 300,000 to 30,000 in 12 days. The coagulation accelerators carried by the platelets become deficient, but the quantity of prothrombin and other accelerators in the blood seems to remain normal. Sometimes there is a spectacular increase in the coagulation time, and sometimes very little. According to ALLEN *et al.*<sup>1</sup> the decrease in coagulability may occasionally be due to the

---

\* Treatment means intervention *after* an incident, e.g. irradiation. The effect of protective substances used before irradiation is dealt with in the following chapter (p. 290).

## PHYSIOPATHOLOGY AND RADIATION SICKNESS IN MAMMALS

presence of an abnormally large quantity of heparin in the blood ; it certainly disappears more or less completely, sometimes even completely, after the injection of protamine or toluidine blue, which are specific antagonists of heparin. The effect of toluidine blue, if it appears at all, is more lasting (24 to 36 hours) than that of protamine (2 hours). It is suspected that molecular changes in certain proteins which intervene in the process of coagulation may play some part, but no precise facts are known. It is certain that blood transfusion, which is effective against the anaemia, has no apparent effect on the haemorrhages, and does not alter either the thrombopenia or the leucopenia. A striking increase of urinary and serum fibrinolytic activity has been observed in irradiated dogs during the three or four days preceding death<sup>29</sup>.

According to CRONKITE *et al.*<sup>23</sup> the important fact is the lack of platelets. Many authors deny that heparin or heparinoid substances play any part in the pathogenesis of the incoagulability of the blood, although the mastocytes, which produce heparin, are radiosensitive and seem to disintegrate after irradiation, setting free their contents<sup>30</sup>. Cronkite's theory is supported by the experimental observation that platelet transfusion \* restores retraction of the clot in irradiated dogs and stops the internal haemorrhages. This is a somewhat academic observation which can only be applied to man in exceptional circumstances, and certainly not in war time, when it is expected that the supply of blood for the treatment of burns and wounds alone would be quite insufficient.

Infection and ulceration accelerate the appearance of the haemorrhages and aggravate them. The capillary, petechial haemorrhages, which are easily visible in the subcutaneous tissues of the mouse, in which the large haemorrhages are less spectacular than in the dog, are easily stopped by adrenochrome semicarbazone<sup>10, 11</sup>. After several investigations, rutin and various flavonoids are regarded as being of no use (see reference 2, p. 206) in treating this condition.

*Infection* in irradiated animals and human beings is a problem of the first importance. When there are no burns or wounds, it is often of intestinal origin, as is shown by the nature of the micro-organisms isolated by blood culture. After irradiation, the body loses some of its ability to form antibodies, it no longer reacts to tuberculin and becomes a prey to micro-organisms which are normally saprophytic. This weakness of the irradiated body must be regarded as one aspect

---

\* By the use of a chelating agent, disodium-ethylene-diamine tetra-acetate at a concentration of 1 per cent and of silicone coated flasks, platelets can be separated by fractional centrifugation without agglutinating<sup>24</sup>.

#### THE SECOND STAGE OF RADIATION SICKNESS

of the diminution of protein synthesis. After an atomic attack, the surgeon will see even insignificant wounds become infected, in spite of careful *débridement*, unless the patients have been treated with antibiotics. Tendency to haemorrhage, the poor state of nutrition, and the defective regenerative powers can only retard healing. Thus, in irradiated patients the slightest wound becomes very serious.

The infection is often of gastro-intestinal or buccal origin because the mucous membrane becomes thin, and loses its physical and biochemical resistance; the fact that it becomes ulcerated at many points makes it the more easily penetrable.

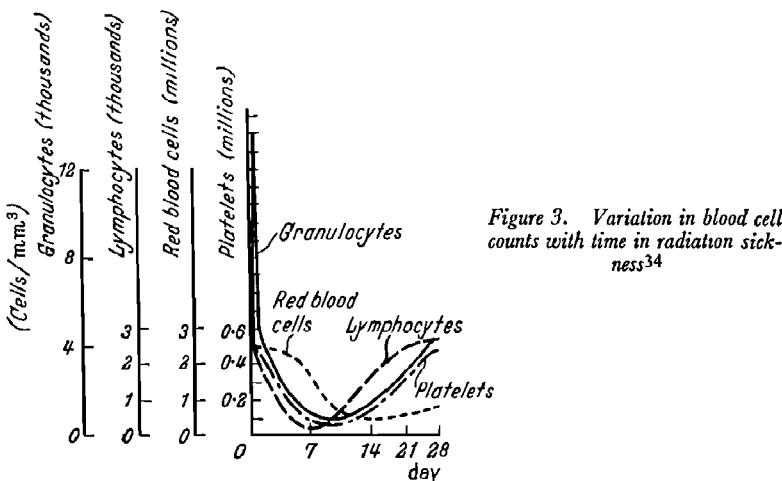
Many animal experiments were carried out, especially in dogs, but also in rats, to discover whether antibiotics prolong the life of irradiated animals, which antibiotic or combination of antibiotics is most efficient, and what dose should be given and when. Provisionally the results may be summarized as follows: In rats, aureomycin orally (or by injection) is excellent; chloramphenicol or combined streptomycin and penicillin are less effective. These antibiotics lower the death rate and prolong the life of the animals which die in spite of treatment. Rats usually die from diarrhoea; after aureomycin treatment the diarrhoea disappears, and the animal dies later from haemorrhage. Aureomycin cannot save the life of any dog which has received at least 450 r, but it prolongs life, diminishes the loss of weight, and slightly reduces the fall in the number of leucocytes. Terramycin lowers the death rate in dogs after 350 r, but the reason for this is not clear. CRONKITE (reference 2, p. 208) thinks that antibiotics should not be used in man until about the fifth to seventh day after irradiation, unless burns, wounds or ulcers make it necessary to intervene earlier. In view of the great variety of micro-organisms which may infect the victim of irradiation, Cronkite recommends, for man, the simultaneous use, orally if possible, of penicillin (1 tablet of 100,000 units every 4 hours) and aureomycin (250 mg every 6 hours). Later, when infections have developed, procaine penicillin (300,000 units a day parenterally) is recommended.

*Anaemia* always develops if the animal lives long enough after irradiation, that is, if it does not succumb to the shock, haemorrhage or gastro-intestinal disturbances. In a dog which has received 450 r, anaemia develops between the second and third weeks. Three factors contribute to its genesis: (i) Cessation of the formation of young erythrocytes by the bone-marrow, which becomes inactive, as is shown by the disappearance of reticulocytes from the circulating blood, and which occurs early, long before the anaemia

## PHYSIOPATHOLOGY AND RADIATION SICKNESS IN MAMMALS

develops; the reticulocytes are young erythrocytes which mature in the plasma in a few days; (ii) haemorrhages of varying severity; (iii) increased destruction of the irradiated erythrocytes; no haemolysis is seen. Nucleated cells appear in the blood of irradiated goats and pigs. The reappearance of reticulocytes is generally a good prognostic sign. Some authors have reported considerable disturbances in the permeability of the red corpuscles, but we have not observed anything on this point in dogs. *Figure 3* summarizes the general variation in blood cell counts in radiation sickness.

Repeated transfusions of whole blood are effective in the treatment of this anaemia, though it has not been shown that they can save the life of an animal which has received a fatal dose of irradiation. The



*Figure 3. Variation in blood cell counts with time in radiation sickness<sup>34</sup>*

effect of homogenates of spleen and bone-marrow will be described in Chapter 16.

It is obvious that in an atomic disaster there will not be enough trained personnel, or material, or blood for transfusion, for the ideal treatment of victims of burns and irradiation. Each case of irradiation (about 300 r) would need about 350 cc of blood in the first week and then every two days to maintain the number of red corpuscles at about 3 million/mm.<sup>3</sup>\* A stay at a high altitude or in a low pressure chamber does not seem to accelerate erythropoiesis in irradiated animals. The cessation of infection and haemorrhage, and the disappearance of fever are the forerunners of a return to normal. Folic acid, cobalamin (vitamin B<sub>12</sub>) and liver extracts

---

\* Assuming an average of 20 days' treatment, and 10,000 victims of irradiation, 35,000 litres of blood would be necessary for a single slight atomic attack.

#### THE SECOND STAGE OF RADIATION SICKNESS

should not be relied upon too much, for the bone-marrow is no longer capable of responding; it needs other substances (see p. 341).

The *malnutrition* produced by irradiation has a complex pathogenesis and is not the same in rats as in dogs, the animals most studied in this respect. It makes metabolic studies in irradiated animals very difficult, for it is necessary either to make individual measurements (food actually ingested, faeces, urine *etc.*) or to starve the controls and the irradiated animals, which necessarily limits the duration of each experiment.

Dogs generally eat well for a week or two after irradiation (450 r) except on the first day. Loss of appetite, vomiting, diarrhoea and loss of weight set in later, when the gastro-intestinal ulcers form. As death approaches the dog takes nothing but water. The gastro-intestinal tract is often paralysed and distended, and contains a large quantity of matter\*.

Doses of 40 to 120 r to the whole body do not affect the appetite, weight and behaviour of dogs. In this species a dose of 200 r seems to be the threshold for radiation sickness, causing a slight loss of appetite for three days.

The gastro-intestinal mucosa is very radiosensitive, as Regaud, Nogier and Lacassagne observed as long ago as 1912. P. Dustin uses this fact in preference to any other test. The damage here is already considerable a few hours after irradiation (cessation of mitosis, pyknosis *etc.*), but repair also sets in very quickly in some mammals. In mice, for example, regeneration is evident three days after irradiation, and in this species death often occurs after the intestinal mucous membrane has recovered.

A growing rat which has received a sublethal dose of x-rays (400 r) often shows loss of weight in two stages. The first loss occurs in the first days after irradiation, and is soon overcome, and the second sets in about 12 days after irradiation and produces a break of about 10 to 15 days in the regular growth curve of the animal. LAMERTON *et al.*<sup>15</sup>, who made this observation, do not explain it. A protector (*e.g.* cysteamine) injected before irradiation diminishes the first loss of weight and abolishes the second. The rat takes less food for the first three days after irradiation (150 to 500 r), the minimal day being usually the day of irradiation. Mice also lose about 10 per cent of their weight in the first 24 hours after a lethal dose of 700 r (see *Figure 14*, p. 316).

Forced feeding is of no use in irradiated animals, because the

---

\* In irradiated rats the weight of the contents of the stomach and intestine sometimes amounts to 10 per cent of the weight of the body<sup>17</sup>.

## PHYSIOPATHOLOGY AND RADIATION SICKNESS IN MAMMALS

gastro-intestinal mucous membrane is able to utilize only small amounts of even predigested food. In rats, forced feeding increases the death rate<sup>18</sup>. Parenteral injections of protein hydrolysate can at the most delay slightly the first loss of weight of rats<sup>12</sup>. Blood transfusions and injections of plasma must be regarded as a form of parenteral feeding. It makes no appreciable difference to the reaction of rats to irradiation whether they are given a diet rich or poor in proteins, with or without the addition of methionine or cystine, before and after irradiation (450 to 550 r)<sup>19</sup>. Vain attempts have been made to improve the state of the digestive tract in irradiated rats by using the standard remedies, atropine, methyl neostigmine bromide, tetraethylammonium and dibenamine<sup>7</sup>; atropine in doses of 300 to 500 mg/kg increases the death rate.

The *late effects* are always the same in radiobiology. There is more or less prolonged sterility in male and female animals which have overcome the toxic phase, the anaemia and the infections. In certain conditions the sterility may be permanent in females, all the oocytes having been destroyed (DESAIVE, BACQ and HERVE<sup>8</sup>). Cataract is frequent, as are lymphomas, especially in mice. These lymphomas are homologous with the human leukaemias. KAPLAN<sup>13</sup>, who has made a detailed study of these tumours in mice has demonstrated the importance of whole-body irradiation. The production of lymphomas is one of the manifestations of the carcinogenic action of ionizing radiations.

## REFERENCES

- <sup>1</sup> ALLEN, J. G., MOULDER, P. V. and EMERSON, D. M., *J. Amer. med. Ass.*, 1951, **145**, No. 10
- <sup>2</sup> *Atomic Medicine*—published by C. F. Behrens, Williams and Wilkins, Baltimore, 1953, 2nd edn, p. 632. See especially the chapters edited by Cronkite
- <sup>3</sup> BACQ, Z. M., FIRKET, J. and HERVE, A., *Bull. Acad. Méd. Belg.*, 1947 6th series, **12**, 295
- <sup>4</sup> BACQ, Z. M., NEUJEAN, G. and VAN LOOY, G., *ibid.*, 6th series, **13**, 351
- <sup>5</sup> BACQ, Z. M., HERVE, A. and SCHERBER, F., *Arch. int. Pharm. Thér.*, 1953, **94**, 93
- <sup>6</sup> BURN, J. H., KORDIK, P. and MOLE, R. H., *Brit. J. Pharmacol.*, 1952, **7**, 58
- <sup>7</sup> DETRICK, L. E., RHODES, B., DEBLEY, V. and HALEY, T. H., *J. Amer. pharm. Ass.*, 1953, **42**, 296
- <sup>8</sup> DESAIVE, P., BACQ, Z. M. and HERVE, A., *Experientia*, 1952, **8**, 436
- <sup>9</sup> FURTH, F. W. *et al.*, *Univ. Rochester Atom. Energy Proj.*, UR-221, 11 August, 1952
- <sup>10</sup> HERVE, A., *Arch. int. Pharm. Thér.*, 1951, **85**, 242
- <sup>11</sup> HERVE, A. and LECOMTE, J., *Arch. int. Pharm. Thér.*, 1949, **79**, 109

#### REFERENCES

- <sup>12</sup> JENNINGS, F. L., *Proc. Soc. exp. Biol. Med.*, 1952, **80**, 10
- <sup>13</sup> KAPLAN, H. S., *Acta Un. int. Cancer*, 1952, **7**, 849
- <sup>14</sup> KOCH, H. J., *Arch. int. Physiol.*, 1954, **62**, 136
- <sup>15</sup> LAMERTON, L. F., ELSON, L. A., HARRIS, E. B. and CHRISTENSEN, W. R., *Brit. J. Radiol.*, 1953, **26**, 510, 568
- <sup>16</sup> LOUTIT, J. F., 'Biological action of radiation', *Lectures on the scientific basis of medicine*, 1952, **1**, 379
- <sup>17</sup> MOLE, R. H., *Brit. J. Radiol.*, 1953, **26**, 234
- <sup>18</sup> SMITH, W. W., ACKERMANN, I. B. and SMITH, F., *Amer. J. Physiol.*, 1952, **168**, 382
- <sup>19</sup> SMITH, W. W., ACKERMANN, I. B. and ALDERMAN, I. M., *ibid.*, 1952, **169**, 491
- <sup>20</sup> TROWELL, O. A., *J. Path. Bact.*, 1952, **64**, 687
- <sup>21</sup> BIGELOW, R. R., FURTH, J., WOODS, M. C. and STOREY, R. H., *Proc. Soc. exp. Biol. Med.*, 1951, **76**, 734
- <sup>22</sup> WEBER, R. P. and STEGGERDA, F. R., *ibid.*, 1949, **70**, 261
- <sup>23</sup> CRONKITE, E. P., JACOBS, G. J., BRECHER, G. and DILLARD, G., *Amer. J. Roentgenol.*, 1952, **67**, 796
- <sup>24</sup> DILLARD, G. H. L., BRECHER, G. and CRONKITE, E. P., *Proc. Soc. exp. Biol. Med.*, 1951, **78**, 796
- <sup>25</sup> LOURAU-PITRES, M., *Ark. Kemi.*, 1954, **7**, 211
- <sup>26</sup> HEVESY, G. and FORSSBERG, A., *Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955
- <sup>27</sup> HALPERN, B. N., CUENDET, A. and MAY, J. P., *J. suisse Méd.*, 1942, **82**, 1020
- <sup>28</sup> HERVE, A. and LECOMTE, J., unpublished observations
- <sup>29</sup> CALGAN, J., GATES, E. and MILLER, J. J., *Univ. Rochester Atom. Proj.*, UR, 189, 25th Oct. 1951
- <sup>30</sup> SMITH, D. E. and LEWIS, Y. S., *Proc. Soc. exp. Biol. Med.*, 1953, **82**, 208
- <sup>31</sup> HALEY, T. H., *Indust. Med. Surg.*, 1953, **22**, 12, 569
- <sup>32</sup> PATT, H. M., *Annu. Rev. Physiol.*, 1954, **16**, 51
- <sup>33</sup> LOUTIT, J. F. and MAYCOCK, W. d'A., *Practitioner*, 1950, **105**, 607
- <sup>34</sup> *The effects of atomic weapons*, p. 348 (*U.S. Atom. Energy Comm.*), McGraw Hill Book Co., New York, 1950

## CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

IN the days when the target theory alone was the foundation of radiobiology it appeared impossible to influence the action of ionizing rays by means of chemical substances.

When the intervention of free radicals and peroxides in an aqueous medium was established, many publications appeared showing that various chemical substances are capable of protecting living organisms against x-rays\*. As early as 1949-50 several independent series of investigations (by the Liège school, Patt and his collaborators, Mole *et al.*) stated the problem correctly. Since then, more than one hundred papers have been devoted to this question, several very useful and well documented reviews have been published, from which the reader will obtain a very complete bibliography and a detailed exposition of the often conflicting views of specialists<sup>1-10</sup>.

To facilitate discussion, the protective substances are divided into the following groups; cyanides and related substances, thiol compounds, substances causing anoxaemia, amines and substances containing an NH<sub>2</sub> group, chelating agents, and miscellaneous substances.

### TECHNIQUES FOR THE STUDY OF PROTECTIVE SUBSTANCES IN MAMMALS

To make a valid study of chemical protection against radiations, it is necessary to have a pure strain of mice or rats, fed on a complete and uniform diet. The irradiation must be lethal for all controls. We have already strongly emphasized this point<sup>2, 14</sup>, following BONET-MAURY and PATT<sup>15</sup>. Many authors determine an LD<sub>50</sub>. This is justifiable in pharmacology, but does not rest on any experimental foundation in radiobiology. With any dose less than the minimum 100 per cent lethal dose, the variations in mortality are

---

\* All that concerns the *treatment* of irradiated animals is being ignored in this chapter. The results obtained with antibiotics, anti-haemorrhagic drugs, adrenocortical hormones, factors promoting regeneration of the haematopoietic system, transfusions, feeding *etc.*, are summed up in Chapters 13 and 16. The effects of anoxia are discussed in Chapter 8.

## STUDY OF PROTECTIVE SUBSTANCES IN MAMMALS

such\* that serious statistical analysis with a large number of animals is essential. A strict technique, illustrated in Figures 1 and 2 is preferred, and one with which it is permissible to avoid statistical analysis. The animals must be irradiated in groups, preferably without anaesthesia, in well standardized conditions. Controls must be inserted in each series to prove that a dose is being used which will kill all controls.

### THE PROTECTIVE SUBSTANCES

*Cyanide*—Sodium or potassium cyanide, in doses that are non-lethal, but sufficient to produce toxic symptoms (0.1 mg for a mouse weighing 20 g) protects 50 to 80 per cent of mice against the mini-

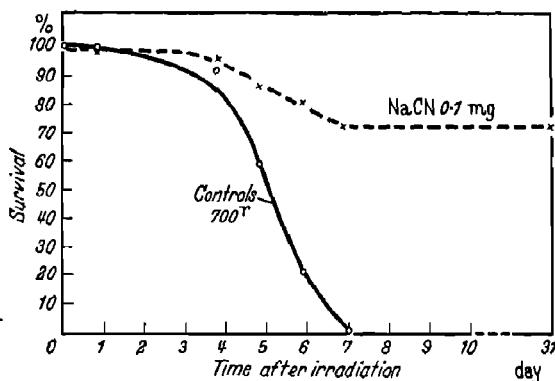


Figure 1. Protective action of cyanide. Survival of control irradiated mice (C57 black) (700 r x-rays, 250 kV) and of cyanide-injected animals (0.1 mg NaCN) similarly irradiated. The injection was intraperitoneal and performed immediately before irradiation<sup>18</sup>.

mum dose lethal for all controls<sup>1, 17-19</sup> (Figure 1). Malononitrile, which liberates cyanide in the body, is a good protector; thiocyanate and cyanate are ineffective. Cyanide injected after irradiation cannot save the life of mice; the molecule must be present in the body during irradiation, and this condition described by Bacq *et al.* in 1949, applies to all the substances mentioned in this chapter. The protective effect of cyanide and nitriles has been confirmed by a number of workers<sup>21-23, 57, 124</sup>, but Dowdy *et al.*<sup>24</sup>, and Patt and Swift<sup>36</sup> failed to find it with rats and frogs respectively. The protective effect of cyanide is additive to that of glutathione<sup>1, 18</sup> (Figure 2), but it has been claimed not to that of cysteine<sup>23</sup>. Cyanide protects *Escherichia coli* against u.v. light<sup>25</sup>.

\* For instance, in several batches of 25 mice of a pure strain, the mortality from the LD<sub>50</sub> of x-rays is sometimes 84 per cent and sometimes 24 per cent<sup>16</sup>.

### CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

Sodium azide which, like cyanide, inhibits cytochromeoxidase and heavy metal enzymes (catalase, peroxydase, catecholoxydase etc.) has a slight protective effect in mice<sup>1, 18, 19, 26</sup>. It lowers the mortality resulting from u.v. irradiation in various micro-organisms, but increases the frequency of mutations in *Micrococcus pyogenes*<sup>27</sup>. BOYLAND and GALLICO<sup>26</sup> observed a relationship between the inhibition of catalase and the protective effect against irradiation (e.g. hydroxylamine which is as effective as azide as a catalase poison, increases the radioresistance of mice<sup>26</sup>). Formate, another inhibitor of catalase, is a radio-protective substance, whereas acetate is ineffective, both as protector against radiation and as an inhibitor

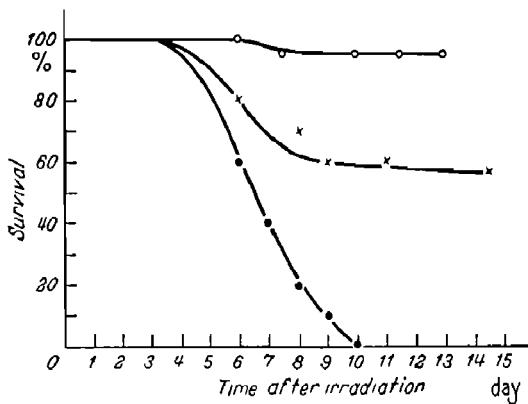


Figure 2. Additive protection of glutathione and cyanide<sup>18</sup>.  
 ● Control mice (700 r)      × 2 mg glutathione injected  
 ○ 2 mg glutathione + 0.1 mg NaCN injected

of enzymes<sup>2</sup>. Cyanide is certainly of no practical use, but the discovery of its radio-protective action has great theoretical importance; it is the key of a series of radio-protective substances whose action must be explained by any general theory. The quantity of NaCN injected into mice is too small to reduce a significant number of —S—S— bridges to —SH.

Cyanide is said to increase the effect of radiations on certain cancer cells<sup>37</sup> and on the roots of *Vicia faba*<sup>38</sup>, although this finding has not been confirmed by READ<sup>152</sup>. Under well defined experimental conditions cyanide protects *Pisum sativum*<sup>39</sup> and ciliates<sup>32</sup>, and also protects barley against the mutagenic effect of x-rays<sup>40</sup>.

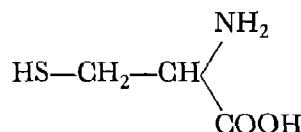
MIKAELSEN<sup>154</sup> finds that cyanide (as well as cysteine and sodium hyposulphite ( $\text{Na}_2\text{S}_2\text{O}_4$ )) decreases the number of chromosome abnormalities produced by  $\gamma$ -rays in growing roots of *Tradescantia paludosa*. In CRABTREE's<sup>37</sup> experiments with tumour cells, which

## THE PROTECTIVE SUBSTANCES

have been confirmed by HALL<sup>151</sup> the sensitizing action of cyanide may be due to an increase in oxygen tension in the tissue as a result of the almost complete inhibition of oxygen consumption; the protective action of cyanide is then outweighed by the increase in radiosensitivity due to this oxygen effect.

The effect of  $\alpha$ -tocopherol, the physiological antioxidant agent for fats, is discussed on p. 234

*Cysteine and glutathione*—Patt and his collaborators, starting from Barron's investigations, have shown that cysteine,

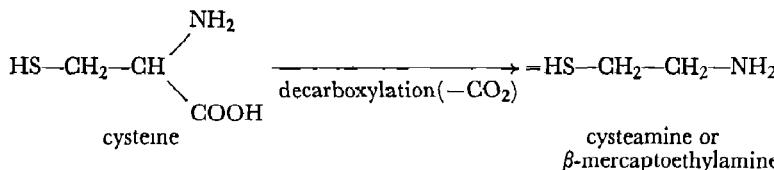


a sulphhydryl-containing amino-acid, protects rats against radiations<sup>28, 29</sup> when injected intravenously or ingested. Cysteine is ineffective if injected a few minutes after irradiation has ended.

Glutathione, a physiological tripeptide containing one molecule of cysteine, is also a good protector provided it is injected and in the reduced state; it is ineffective when ingested<sup>16, 29, 47, 48</sup>.

Methionine, an amino-acid which contains an  $-\text{SCH}_3$  group is inactive, although its methyl group is regarded as very labile<sup>2, 29</sup>; homocysteine, i.e. demethylated methionine, containing a free  $-\text{SH}$  group, has relatively little effect<sup>2</sup>,  $\text{Na}_2\text{S}$  is ineffective<sup>29</sup>, but the hydrosulphide ( $\text{NaSH}$ ) is a good protector for bacteria<sup>120</sup>. The protective effect of cysteine and glutathione against radiations has been confirmed many times, whatever biological material has been used (mammals, isolated normal or cancer cells, plants, micro-organisms)<sup>1, 4-8, 14, 16</sup>.

*Cysteamine and cystamine\**—As long ago as 1951 Bacq and his collaborators discovered the remarkable protective action of  $\beta$ -mercaptopethylamine (= Bepantan Labaz), also known as cysteamine because it is the amine corresponding to cysteine<sup>50</sup>.



In non-toxic doses cysteamine reduces the effects of x-rays by about one-half. Mice which have received 150 mg/kg of cysteamine

---

\* In accordance with the terminology adopted by BACQ *et al.* (*Science*, 1954, **119**, 163).

CHEMICAL PROTECTION AGAINST X- AND  $\gamma$ -RAYSTable I. Protection of Mice by sulphhydryl compounds\* against 700 r of x-rays<sup>2</sup>

Substance			Number of Mice surviving out of 10
$\beta$ -Mercaptoethylamine (cysteamine)	.	.	9.7†
Cysteine	.	.	3
Homocysteine	.	.	5
Glutathione	.	.	{ 4 5 ( $0.7 \times 10^{-5}$ moles) 0
Thioacetamide‡	.	.	{ 0 ( $2 \times 10^{-5}$ moles) 1 to 2
Mercaptosuccinic acid	.	.	0 ( $1 \times 10^{-5}$ moles)
$\alpha$ -Thiobenzoic acid§	.	.	0
Thioethanol	.	.	0
Ergothioneine	.	.	{ 0 0 ( $2 \times 10^{-5}$ moles)
Thioacetic acid (Na salt)	.	.	0
Mercaptothiazoline	.	.	4
Mercaptobenzothiazol	.	.	10
Derivatives of Cysteamine			
$n$ -Pantothenylcysteamine (pantetheine)	.	.	{ 0 ( $0.8 \times 10^{-5}$ moles) 1
Cystamine†	.	.	10 ( $2 \times 10^{-5}$ moles)
$sn$ -Diacetylmercaptoethylamine	.	.	1 to 9
s-Acetylmercaptoethylamine	.	.	0 to 2
s-allylcysteamine	.	.	0 ( $1 \times 10^{-5}$ moles)
s-methylcysteamine	.	.	{ 0 3 ( $12 \times 10^{-5}$ moles)
$\beta$ -aminoethyl sulphuric acid	.	.	1

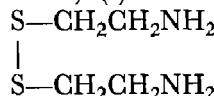
\* The dose injected into the mice corresponded to  $4 \times 10^{-5}$  moles.

† Out of 100 mice irradiated in ten batches of ten mice each 97 animals survived.

‡ Toxic; with 2.9 mg 5 animals dead on the third day, 7 on the fourth, the 3 remaining, dead before the controls. The dose of 1.45 mg is well tolerated; 6 mice still living on the tenth day when all the controls are dead; no survival at the fifteenth day.

§ Toxic,  $4 \times 10^{-5}$  moles kills all mice rapidly.

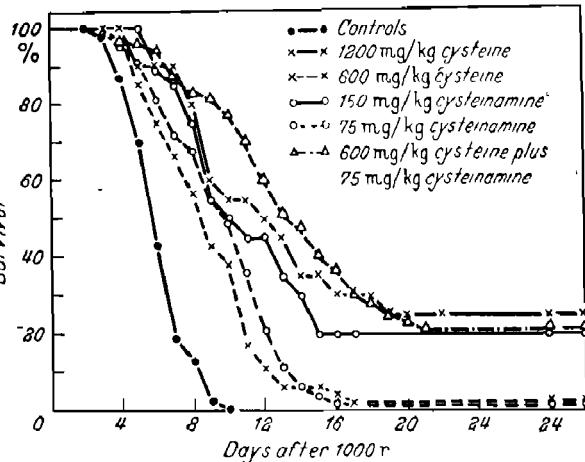
intraperitoneally need a dose of x-rays twice as large as controls do to produce the same mortality (Figures 3 and 4). According to BACQ and HERVE<sup>14, 43</sup> the high activity of cysteamine is only an example of a general law well known to pharmacologists, that amines are more active than the corresponding amino-acids, the disappearance of the —COOH group 'setting free' the amine function. Cysteamine has many advantages: (a) It is more easily and more cheaply synthesized than cysteine; (b) in equimolecular quantities, it is about five times as effective as cysteine<sup>14</sup> (confirmed by STRAUBE and PATT<sup>125</sup>); its protective action has been confirmed by many other workers<sup>89, 91-94</sup>; (c) the corresponding disulphide, cystamine:



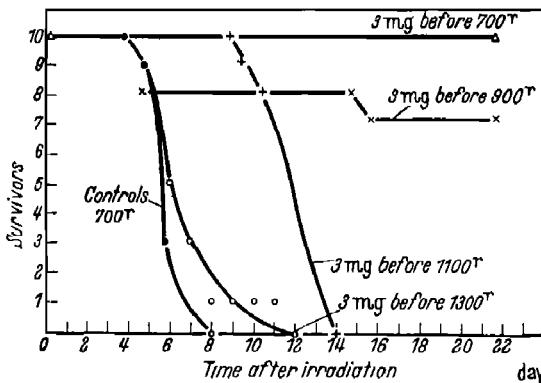
### THE PROTECTIVE SUBSTANCES

unlike cystine, is an excellent protector, even a little better than cysteamine<sup>14, 50</sup>; it is active when administered orally, and is very well tolerated by the gastro-intestinal tract<sup>58</sup>, it is chemically completely stable, which is not true of cysteine and cysteamine.

**Figure 3.** Synergistic effect of cysteinamine and cysteine upon survival of mice following exposure to 1000 r total-body irradiation. Figures in parentheses indicate number of animals in each group. 1000 r is far beyond the 100 per cent threshold lethal dose<sup>125</sup>



Cysteamine is also of great interest in general biology, because it is the portion of the co-enzyme A molecule which carries the —SH group, where all the exchanges take place (see p. 242). These facts have given rise to a series of biochemical investigations which we



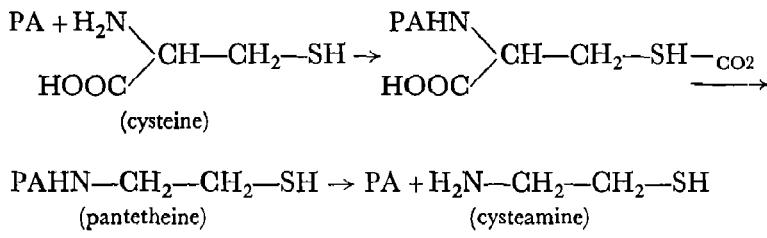
**Figure 4.** Protection of 20 g mice by intraperitoneal injection of 3 mg cysteamine immediately before irradiation with various doses of x-rays<sup>69</sup>

summarize here because they may have considerable importance in radiobiology.

(i) Cysteamine cannot be incorporated in proteins nor be used in the synthesis of CoA<sup>59</sup>, it is a general rule that amines cannot be used as such in the growth of living organisms.

## CHEMICAL PROTECTION AGAINST $\alpha$ - AND $\gamma$ -RAYS

(ii) The decarboxylation of cysteine is supposed to take place after the union of this amino-acid with pantothenic acid (PA).



The pantetheine thus synthesized is a growth factor well known to microbiologists (*Lactobacillus bulgaricus* factor =LBF); a hepatic enzyme may liberate cysteamine from pantetheine. Consequently

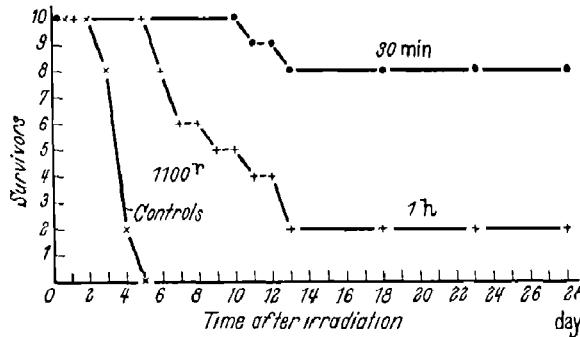


Figure 5. Survival of two series of mice irradiated (1100 r x-rays on whole body) after ingestion of 400 mg/kg cystamine 2HCl compared with unprotected controls<sup>58</sup>

the liver and perhaps other tissues, would always have a certain amount of cysteamine available.

(iii) The metabolism of cysteamine and of cystamine is rapid. It has been followed by means of molecules labelled with  $^{35}\text{S}$  (see *Table II* and *Figure 6*). A part is excreted unchanged; this fraction is important if the molecules are administered intravenously, intra-peritoneally or intramuscularly<sup>60, 62, 64-66</sup>. The liver concentrates and oxidizes cysteamine into  $\text{SO}_4^-$  ions and taurine, which is partly excreted in the urine and partly combined with cholic acid by the bile<sup>60-62</sup>. The bone-marrow is believed to concentrate cysteamine<sup>67, 162</sup> though the distribution in the mammalian body is very irregular, and is probably even more so when these amines are administered orally.

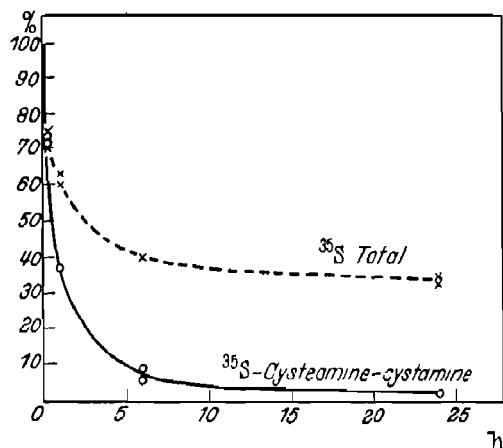
### THE PROTECTIVE SUBSTANCES

*Table II. Total  $^{35}\text{S}$  Activity in different Tissues after Intraperitoneal Injection of Cysteamine (3.14 mg/20 g mouse)<sup>61</sup>*

Time after injection:	Radioactivity as counts/min/g of tissue $\times 10^5$						
	15 min	1 h	6 h	24 h			
Mouse (No.)	1	2	3	4	5	6	7
Blood . . .	1.14	1.11	0.69	0.69	0.18	0.14	0.15
Liver . . .	5.10	4.55	4.38	4.34	5.60	2.86	3.54
Intestine + pancreas . . .	3.78	2.98	2.22	3.04	2.55	3.24	3.46
Kidney . . .	5.37	6.08	4.00	3.31	1.47	1.73	2.05
Brain . . .	1.81	1.55	1.57	1.83	0.27	0.30	0.31
Remainder . . .	1.94	1.63	1.44	1.63	0.80	0.80	0.63

(iv) Cystamine ( $-\text{S}-\text{S}-$ ) is reduced to cysteamine ( $-\text{SH}$ ) both *in vivo*<sup>64</sup> and *in vitro*<sup>65</sup>, whereas cystine is not reduced *in vivo*<sup>64</sup>. This may explain, to some extent, why cystamine is an effective protector whereas cystine is not, but it does not alter the fact that excellent protection is given by a substance which contains sulphur but does

*Figure 6. Comparison of total  $^{35}\text{S}$  activity to activity of  $^{35}\text{S}$  cysteamine-cystamine in the whole body of mice at various times after intraperitoneal injection of 3 mg  $^{35}\text{S}$  cysteamine. (Cystamine and cystine cannot be separated, the technique involves crystallization of cystamine present as such and formed by oxidation of cysteamine<sup>165</sup>)*



not supply the body with any  $-\text{SH}$  groups. By extension to pantetheine and CoA, it may be assumed that the oxidation-reduction potentials *in vivo* are such that these two substances must remain in the reduced state. This is important, because they lose all their enzymatic activity when they are oxidized to  $-\text{S}-\text{S}-$ .

(v) Cystamine (unlike cysteamine), when injected intravenously or subcutaneously, liberates histamine because it has two  $-\text{NH}_2$  groups<sup>68, 69</sup>. It cannot therefore be injected in medical practice, but this disadvantage is obviated if cystamine is given orally, because any histamine eventually liberated is stopped by the intestinal

## CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

mucosa and the liver<sup>58</sup>. Since cystamine retains all its protective action against radiations when given orally, it must be concluded, in opposition to PATT<sup>4</sup>, that the liberation of histamine is not the cause of its efficacy. Cystamine is rapidly absorbed by the intestinal tract of mice<sup>58</sup> dogs and man<sup>62</sup>.

(vi) Like cysteine<sup>4</sup>, cysteamine protects rats<sup>70</sup> and *Allium* roots<sup>71, 72</sup> against the effects of nitrogen mustards. Cysteamine also protects nerve cells from the toxic effects of streptomycin<sup>73, 74</sup>.

(vii) The observation that cysteamine protects mice against poisoning by oxygen (*i.e.* an environment of 6 atmospheres of oxygen) is of practical as well as theoretical interest<sup>160</sup>. Other similarities between high oxygen tension and x-rays are that the ascorbic acid content of the adrenals is reduced as in stress<sup>161</sup>, and that under certain conditions chromosomes are broken and HO<sub>2</sub> radicals may be produced (see p. 212).

(viii) Cysteamine and cystamine have other actions which may explain the fact that they have a beneficial effect on radiation sickness in patients with cancer or other disease treated by local irradiation, *even if they are administered after irradiation*<sup>75, 76\*</sup>.

(a) Without being antihistaminic, cysteamine and cystamine have a general anti-oedematous and anti-inflammatory effect<sup>77-80</sup>.

(b) In concentrations of 1:20,000 they inhibit the growth of tissue cultures by acting on the cytoplasm<sup>81</sup>; they also inhibit the synthesis of haemoglobin by the reticulocytes of dogs *in vitro*<sup>126</sup>. These facts may explain the favourable effect of cysteamine in certain cases of leukaemia and lymphoid tumours<sup>82, 83</sup>.

(c) Cysteamine and cystamine cause ascorbic acid to leave the adrenal glands<sup>84</sup>; they cause the excretion of a large quantity of ascorbic acid in the urine<sup>64, 69</sup> and the appearance in the blood and urine of reducing substances† which have not yet been identified<sup>64</sup> (*Figure 7*). Cysteamine increases the ascorbic acid content of the liver<sup>86</sup>.

(d) These two amines cause a rapid fall in the glycogen reserves of the liver, without producing hyperglycaemia<sup>85</sup> (*Figure 8*). They do not cause hypersecretion of corticoid hormones<sup>82, 84</sup>. Other protectors against radiations (glutathione, diethylidithiocarbamate) have no similar effect on the glycogen content of the liver<sup>42</sup>.

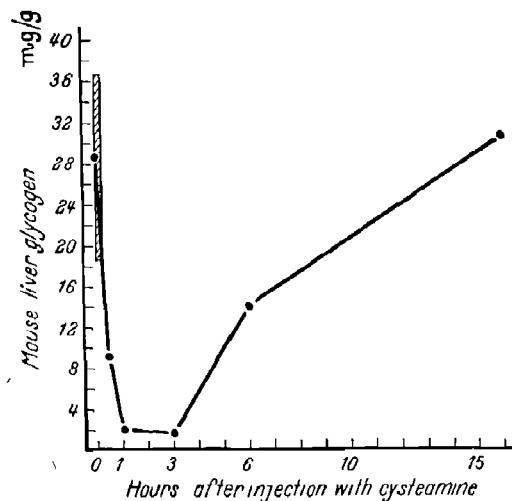
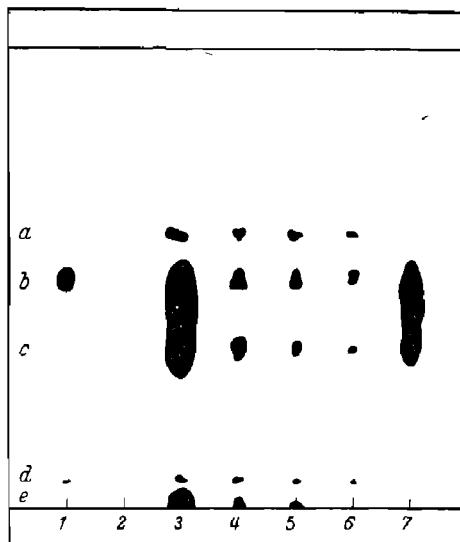
\* PATT<sup>4</sup> has found observations by a Japanese, M. Shirai, according to which glutathione injections are also effective against radiation sickness in man.

† These metabolic reducing substances are unlikely to play a part in the protection, as they persist for a long time in the body while the protection following an injection of cysteamine runs parallel with the concentration of this substance in the body.<sup>14</sup>

### THE PROTECTIVE SUBSTANCES

To sum up, in considering the protective effect of cysteamine and cystamine against radiation injuries we must not forget that these molecules have important metabolic properties by themselves, which

*Figure 7.* Paper chromatograms (developed to show reducing substances) of dog urine after intravenous injection of cysteamine: (1) cysteamine HCl; (2) control urine before injection; (3) urine collected during the first hour after injection; (4) urine of the second hour; (5) urine of the third hour; (6) urine of the fourth hour; (7) cysteamine ascorbate. Spot b is cysteamine, c is ascorbic acid, d is cystamine. The two other spots a and e have not been identified<sup>64</sup>



*Figure 8.* Effect of 150 mg/kg cysteamine injected interperitoneally on glycogen content of mouse liver. The rectangle at zero time indicates the observed variation of glycogen content of the liver of 16 normal mice<sup>42</sup>

are not shared by cysteine or other powerful protectors against radiations<sup>42</sup>. At present in medical practice cysteamine and cystamine offer the best compromise between efficacy, toxicity and cost.

### CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

HALEY, MANN and DOWDY<sup>54</sup> write: 'However, sulphhydryl compounds are of no practical use in irradiation protection of humans, although they are extremely useful as laboratory tests.' This conclusion, which is true for cysteine and glutathione, is false as a generalization. Unlike many sulphur compounds, cysteamine and cystamine do not affect the thyroid<sup>82\*</sup> and are very well tolerated. There are records of hundreds of experiments made in Belgium, France, Great Britain and Scandinavia.

*Other sulphhydryl compounds*—Many —SH compounds even physiological substances give little or no protection against x-rays to mammals (see *Table I*), but are very effective protectors of micro-organisms. Among these are dimercaptopropanol (BAL), hydrosulphite, mercaptosuccinate and mercaptopyruvate<sup>1, 4, 6, 57</sup>.

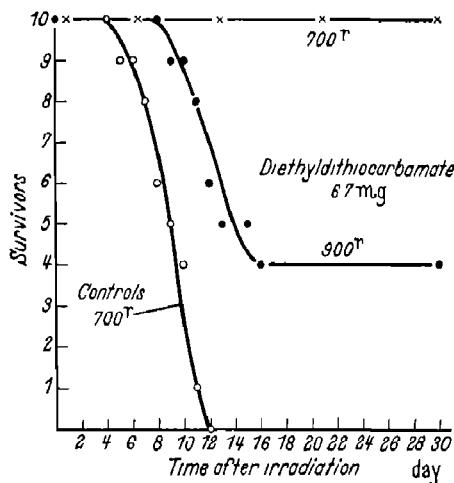


Figure 9. Protection of mice by Na diethyldithiocarbamate<sup>42</sup>

*Chelating agents*—BACQ, HERVE and FISCHER<sup>42</sup> have observed that various chelating agents are remarkably effective protectors of mice against radiations. Unfortunately many of them are very toxic, and our series was reduced to diethyldithiocarbamate (*Figure 9*), ethylenediaminetetraacetic acid (versene or sequestrene) and trihydroxy-N-methylindol. Mercaptoethylamine is an excellent chelating agent since it contains a covalent group (—SH) and a strongly ionized group (—NH<sub>2</sub>) very close together, whence the idea that traces of certain ions play a very important part in the radiochemistry of aqueous solutions. Chelating agents are also among the best protectors in an *in vitro* system in which their mode of

\* According to B. Houssay (personal communication) cysteamine does not affect the uptake of radioactive <sup>131</sup>I by the thyroid.

### THE PROTECTIVE SUBSTANCES

of action has been studied (see p. 314 and *Table III*). Since (i) radiochemical reactions are not altered in the absence of traces of copper, and (ii) the addition of copper to chelating agents suppresses their protective action, it seems that the structure which favours chelation is also that which produces the protective action against radiations<sup>2</sup>.

*Table III. Comparison of Protection of Polymer and of Mice by Miscellaneous Compounds\**

<i>Substance</i>	<i>Per cent protection for polymer</i>	<i>Mice surviving out of 10</i>
<i>Glucose</i>	47	0 to 3 ( $1.5 \times 10^{-3}$ moles)
<i>Fructose</i>	61	8 ( $1.5 \times 10^{-3}$ moles)
<i>Sodium cyanide</i>	80 ( $4 \times 10^{-4}$ M)	5 to 7 ( $2 \times 10^{-6}$ moles)
<i>Malononitrile</i>	38	8 ( $2 \times 10^{-6}$ moles)
<i>Sodium azide</i>	54	4 ( $1.6 \times 10^{-6}$ moles)
<i>Formic acid</i>	37	6
<i>Acetic acid</i>	0	1 to 0
<i>Caprylic acid</i>	54	5
<i>Diethylthiocarbamate</i>	88	10

\* The concentration of the protector in the polymer solution was  $8 \times 10^{-4}$  M, the dose injected into the mice corresponded to  $4 \times 10^{-5}$  moles. The x-ray dose was 700 r and killed 100 per cent of the control animals.

*Alcohols, sugars, substances containing hydroxyl groups*—Simple alcohols (e.g. ethanol), glycols (glycerine, propylene glycol) are feeble protectors both of micro-organisms<sup>6</sup> and of mammals<sup>2, 13, 42, 44, 46, 49</sup>, and so are the sugars<sup>6, 14, 87</sup>, but fructose is better than glucose<sup>2</sup>; the same applies *in vitro* (see *Table III*). Phenols are excellent protectors *in vitro*, but are too toxic to be compared in equimolecular doses with other protectors in mice<sup>2</sup>, although ketonic derivatives of phenols were found by LACASSAGNE *et al.*<sup>140</sup> to protect mammals in non-toxic doses.

*The carboxylic acids*—Formate is a good protector both of the polymer system *in vitro*<sup>2</sup> and of *E. coli*<sup>6</sup> and mice<sup>2</sup>, whereas acetate is ineffective<sup>2, 50</sup>; caprylate and methyl linoleate<sup>111</sup> are effective again (see *Table III*). Pyruvate<sup>6, 101</sup>, succinate,  $\alpha$ -ketoglutarate, citrate and the acids of Krebs cycle in general, protect bacteria<sup>6</sup> but act very irregularly in mice<sup>2, 14</sup>. Pyruvate gives good protection to dog reticulocytes irradiated *in vitro*<sup>33</sup>.

*Amino-acids and amines*—Amino-acids are weak protectors both of *E. coli*<sup>6</sup> and mice<sup>2, 14</sup>. The amines, especially the aromatic amines, are far better protectors than the corresponding amino-acids

CHEMICAL PROTECTION AGAINST X- AND  $\gamma$ -RAYS

(Table IV<sup>14, 43, 95</sup>). Here again a substance with *one* carbon (methylamine) is more active than one with *two* carbons (ethylamine<sup>2, 41</sup>). Among the most interesting<sup>2, 14</sup> are tryptamine,

Table IV. Comparison of Protection of Polymer and of Mice by Amino Compounds\*

Substance	Per cent protection for polymer	Mice surviving out of 10
Glycine . . .	18	0
Glycine ethyl ester . . .	13	
n-Diethylalanine . . .	24	3
Lysine . . .	15	0
Arginine . . .	19	1
Aspartic acid . . .		{ 1 ( $8 \times 10^{-5}$ moles) 0
Glutamic acid . . .	0	0
Cystine . . .	9 ( $4 \times 10^{-4}$ M)	0 ( $2 \times 10^{-5}$ moles)
Methionine . . .	33	0
Histidine . . .	55	0
Phenylalanine . . .	34	{ 3 ( $1 \times 10^{-5}$ moles) 1
Tyrosine . . .	43	0 ( $1 \times 10^{-5}$ moles)
Tryptophane . . .	87	3 ( $1 \times 10^{-5}$ moles)
Ammonium chloride . . .	0	5
Methylamine . . .	10	3 to 7
Ethylamine . . .	12	1
Allylamine . . .	60	0 ( $1 \times 10^{-5}$ moles)
Dimethylamine . . .	11	0
Ethanolamine . . .	15	{ 1 to 3 0 ( $2 \times 10^{-5}$ moles)
Triethanolamine . . .	56	{ 0 2 ( $1.3 \times 10^{-5}$ moles)
Ethylene diamine . . .	11 ( $4 \times 10^{-4}$ M)	3
Choline . . .	17	1 ( $1 \times 10^{-5}$ moles)
d-Glucosamine . . .	30	0
Histamine . . .	23	7†
Aniline . . .	74	1 to 4
p-Aminobenzoic acid . . .	33	{ 0 1 ( $8 \times 10^{-5}$ moles)
$\beta$ -Phenylethylamine . . .	72	{ 2 8 ( $1 \times 10^{-5}$ moles)
Tyramine . . .	59	{ 8 ( $1 \times 10^{-5}$ moles)
Hydroxytyramine . . .	64	{ 7 to 10 10 ( $1 \times 10^{-5}$ moles)
Dihydroxyphenylalanine . . .	46	{ 0 4 ( $1 \times 10^{-5}$ moles)
m-Tyramine . . .	71	10
Tryptamine . . .	76	10 ( $1 \times 10^{-5}$ moles)

\* The concentration of the protector in the polymer solution was  $8 \times 10^{-4}$  M; the dose injected into the mice corresponded to  $4 \times 10^{-5}$  moles. The x-ray dose was 700 r and killed 100 per cent of the control animals.

† Results very variable. See BACQ and HERVE<sup>14</sup>.

### THE PROTECTIVE SUBSTANCES

5-hydroxytryptamine (=serotonin), adrenaline and noradrenaline<sup>2, 14, 43, 88, 90</sup>, because they are physiological substances in vertebrates and in many invertebrates\*.

The toxicity of 5-hydroxytryptamine and noradrenaline prevents the practical use of these powerful protectors. No experiments have yet been made to throw light on the part played in the reaction to radiations by the considerable local concentrations of these amines which exist in certain cells of certain organs or tissues (adrenal medulla, amphibian skin, Kulchitsky cells in the intestines, posterior salivary glands of cephalopods etc.) see<sup>95</sup>.

*Reducing substances without sulphydryl groups*—Ascorbic acid, though it seems to play a part which is not yet well defined, is a weak and very erratic protector<sup>29, 30</sup>. We have not been able to confirm in mice the favourable effect obtained by LOISELEUR and VELLEY<sup>96</sup> in rabbits, from the injection of a large dose of ascorbic acid + cysteine after irradiation. LOISELEUR<sup>144</sup> has also found a complex mixture of glucose, cysteine, thiourea, ascorbic acid and succinic acid to be effective if given either before or after irradiation. Sodium hydro-sulphite( $\text{Na}_2\text{S}_2\text{O}_4$ ) can only be used to study protection against irradiation in bacteria<sup>6</sup> and it is one of the favourite substances of Hollaender's group, who have studied the whole series of salts of the various sulphurous acids. Bisulphite ( $\text{NaHSO}_3$ ) and metasulphite ( $\text{Na}_2\text{S}_2\text{O}_5$ ) are as good protectors as hydrosulphite; bisulphite ( $\text{NaHSO}_3$ ), thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3$ ), dithionate ( $\text{Na}_2\text{S}_2\text{O}_6$ ), pyrosulphate ( $\text{Na}_2\text{S}_2\text{O}_7$ ) and peroxydisulphate have practically no effect<sup>120</sup>; the last three salts are oxidizing substances.

*Methaemoglobin-producing substances and carbon monoxide*—*p*-Amino-propiophenone, in doses which oxidize 75 per cent of the haemoglobin into methaemoglobin, gives a considerable survival rate in rats and mice<sup>88, 97</sup>. Nitrite ( $\text{NaNO}_2$ ) is also a fairly effective, though toxic protector. Its action is additive with that of cysteine, but not with that of ethanol<sup>99</sup>. It is not the methaemoglobin content which determines the degree of protection against radiations; according to COLE and ELLIS<sup>99</sup>, anoxia is not the essential factor in the action of nitrite. Coal gas, thanks to its CO (carbon monoxide) content is, with cyanide and malononitrile, the only really effective protector among the 22 substances tested in mice by BONET-MAURY and PATTI<sup>124</sup>. The mechanism of the action of CO is supposed to be anoxia through the formation of carboxyhaemoglobin; indeed, the protective effect appears when 60 to 65 per cent of the haemoglobin has been converted into carboxyhaemoglobin,

\* Pitressin, a polypeptide vasoconstrictor hormone, also has an appreciable protective action against radiations<sup>88</sup>.

### CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

which is incapable of transporting oxygen. KING, SCHNEIDERMAN and SAX<sup>149</sup> found that the effect of x-rays on *Tradescantia* microspores was increased if the material was exposed to a mixture of air and CO before and during the irradiation. GRAY<sup>150</sup> using CO at pressures up to 50 atmospheres made a similar observation with *Vicia*. The maximum increase in sensitivity, a factor of 1.8, was obtained when the roots were exposed to CO for 30 minutes before as well as during the irradiation with x-rays. CO even at these high pressures does not alter the sensitivity of *Vicia* to neutrons. This latter observation indicates that an explanation may have to be sought in radiochemical terms although no interpretation has yet been given.

*Miscellaneous substances*—A sulphur containing barbituric derivative, pentobarbital<sup>11-13</sup>, triphenyltetrazolium chloride<sup>45</sup>, and morphine<sup>106</sup> give very slight protection to small rodents. Vitamins of the B group (even pteroylglutamic acid, folic acid and colabamine or B<sub>12</sub>) are ineffective<sup>7, 14, 30, 43</sup>. If cobalt (CoCl<sub>2</sub>) is added to the diet of mice for 8 days before and 15 days after irradiation, 60 to 70 per cent of the animals survive irradiation with 720 r<sup>113</sup>. According to LANGENDORFF and KOCH<sup>148</sup> ether and urethane increase the radiosensitivity of mice while barbiturates and megaphen alone have no effect. Megaphen given with dolantine (a mixture which decreases body temperature) was found by these workers to produce radioresistance (*i.e.* give protection) in the same way as ethanol.

Certain oestrogenic steroid hormones have a slight protective effect<sup>121, 145,\*</sup>. The steroids of the adrenal cortex (cortisone *etc.*) have no favourable effect (see p. 272). Testosterone strikingly inhibits the development of lymphoid tumours after total irradiation of mice with 673 r divided into 4 equal doses at 4 days' interval, but this is not a matter of *protection* in the physicochemical sense (competition for free radicals). The testosterone is injected *after* each irradiation, and then twice a week for 10 weeks<sup>122</sup>. This is a long-term effect on the slow process of carcinogenesis which is shared by cortisone, but not by deoxycorticosterone<sup>123</sup>.

There are not many *good* protectors against radiations, that is, substances which, in non-toxic doses, are capable of reducing the effect of x-rays on mammals by about a half. Cysteine (HCl) in very large doses (1 g/kg) and mercaptoethylamine (100 to 150 m/kg of the base) are those which have been best studied. It would be useful to determine the DRF (dose reduction factor) of diethyl-dithiocarbamate<sup>42</sup> and of the few chelating agents which are not toxic for mammals<sup>2, 42</sup>.

---

\* Female mice are slightly more radioresistant than males<sup>146, 147</sup>.

## MECHANISM OF THE ACTION OF PROTECTORS

### MECHANISM OF THE ACTION OF PROTECTORS

Two facts dominate the action of protectors, and will serve as a guide for discussion.

(i) *Protectors\* act only if they are present at the time of irradiation, i.e. at the time when the energy is absorbed, at the moment when the free radicals are active.* It seems that by injecting enormous doses (of the order of 1 g/kg) of cysteine intravenously immediately after irradiation it may be possible to lower the mortality slightly. The quantity of cysteamine which, when injected before irradiation,

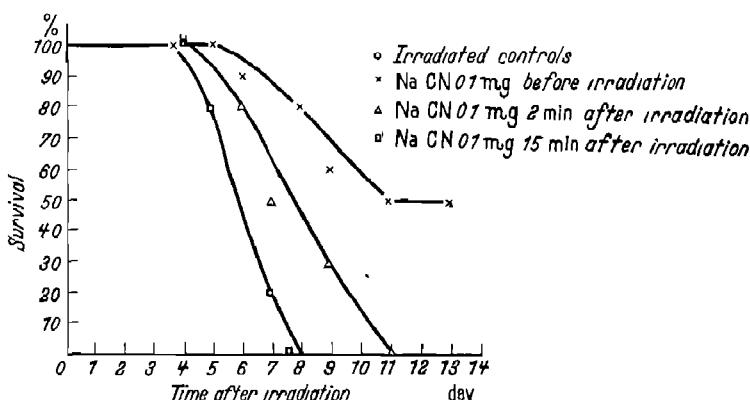


Figure 10. Comparison between administering protectors before and after irradiation. There is no difference between controls and animals injected 15 min after irradiation<sup>19</sup>

halves the effect of x-rays is quite ineffective if injected intraperitoneally in mice within 20 seconds after a lethal irradiation which has lasted only 5 to 25 seconds<sup>30, 31</sup>.

(ii) *Most good protectors for mice are also excellent protectors for a polymer—Polymethylmethacrylate (PMA) which is degraded by ionizing radiations in a medium containing oxygen<sup>2, 100</sup>.* This is demonstrated by Tables III and IV taken from BACQ, ALEXANDER *et al.*<sup>2</sup> and will be discussed in detail on p. 314.

The effect of protectors is universal, and has been confirmed in isolated cells† (bacteria<sup>6</sup>, dog reticulocytes<sup>33</sup>, infusoria<sup>32, 141</sup>,

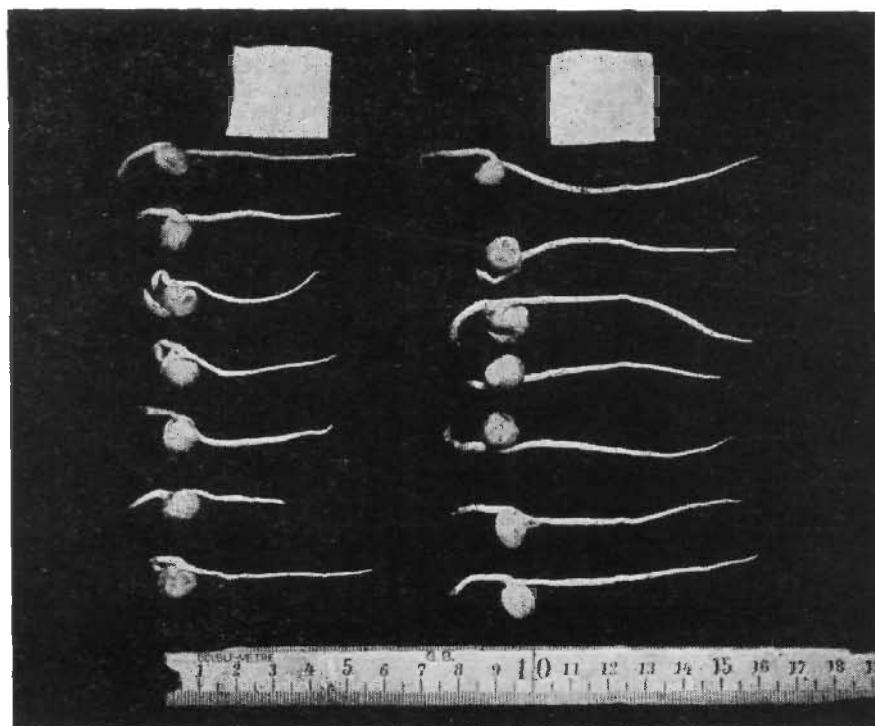
\* Regrettable confusion in terminology is already to be found in the literature. Some authors speak of 'protection' when they inject homogenates of spleen or bone-marrow *after* irradiation. This is pure *therapy* by the replacement of a growth factor, and is no longer protection.

† HOLLOWELL and DOUNREY<sup>139</sup> have made a most detailed study of the protective action of cysteamine and of mercaptoethanol against the effects of x-rays on *E. coli*. With a dose of 60,000 r cysteamine gives a remarkable degree of protection, the dose reduction factor being twelve (*i.e.* the number of organisms killed by 60,000 r in the presence of cysteamine is the same as that by 5000 r in

## CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

isolated thymocytes<sup>34</sup> etc., growing plants<sup>39</sup> (*Figure 11*). It is therefore not possible to invoke some mechanism peculiar to vertebrates, such as a diminution of the stress reaction (see pp. 267-279)\*. It is therefore suitable to discuss the action of the substances mentioned above in relation to only a few possibilities.

*Elimination of invalid hypotheses*—Various hypotheses or suggestions proposed at different times which are in flagrant contradiction with



*Figure 11. Protection of germinating peas. Left, irradiated controls (350 r); right, irradiated after 4 hours' treatment with a soln NaCN 1/4000<sup>39</sup>*

known facts will be ignored, for example (i) the fact that a substance is toxic, or injected in toxic doses, is sufficient to make it a protector

its absence). At higher doses the protection by cysteamine decreases while that of mercaptoethanol increases. A surprising observation is that after irradiation with cysteamine the amount of recovery which occurs by post-irradiation exposure to sub-optimal temperatures (see p. 265) is much less than that of the controls. This is not found with mercaptoethanol. The amount of protection observed with cysteamine depends therefore on the experimental condition used and illustrates the complexities which are seen once these processes are investigated in detail.

\* That is, if the 'stress' reaction is inhibited in vertebrates (see 114), it means that certain cells in the hypothalamic centres or in the pituitary have been protected from the first against x-rays. It is this *cellular* protection which is to be interpreted in a very wide framework. Besides, adrenalectomy diminishes, but does not abolish the protective effect of cysteine<sup>117</sup>.

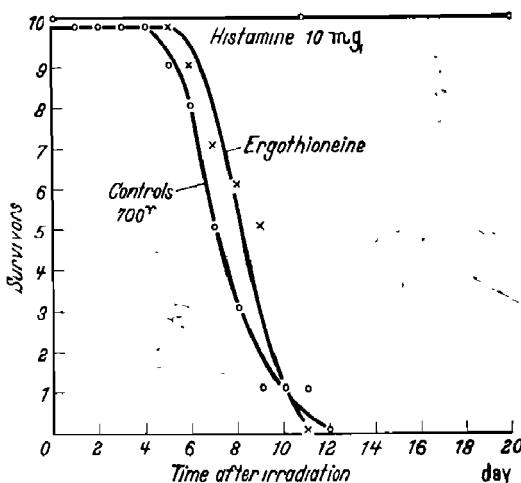
### MECHANISM OF THE ACTION OF PROTECTORS

against radiations<sup>35</sup>. From our experience of more than four years, we can affirm that many toxic substances, injected in toxic doses, have no protective action (NaF, arsenical substances, fluoroacetate, mercuribenzoate etc.).

(ii) The protective action against radiations is supposed to be linked to the reducing property; but many reducing substances, even physiological substances, have little or no effect (ascorbic acid, ergothioneine (*Figure 12*), pantetheine<sup>2, 14, 29</sup>). However, most good protectors for mammals are more or less powerful reducing agents (NaCN, cysteine, glutathione, diethyldithiocarbamate, noradrenaline, 5-hydroxytryptamine etc.).

Many radiobiologists, following Barron, have become enthusiastic about the sulphhydryl group. This question has been discussed in

*Figure 12. Failure of physiological sulphhydryl compound, ergothioneine to protect mice. On the same graph protection by an equimolecular dose of histamine is shown although results with this substance are not consistent*

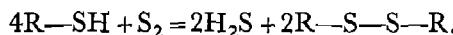


detail by PATT<sup>155</sup> (see also p. 240). Great caution is needed; facts observed *in vitro* with —SH enzymes cannot be assumed true *in vivo*. *In vitro*, the addition of cysteine *after* irradiation is sufficient to reactivate an enzyme which has been partially inactivated by x-rays. *In vivo*, cysteine, glutathione, cysteamine and cystamine are ineffective after irradiation. Many substances supposed to reactivate enzymes with thiols oxidized into —S—S— by ionizing radiations have no protective action against the effects of radiations. Many substances which do not reactivate —SH enzymes inactivated by oxidation into —S—S— are excellent protectors against radiations. *Table I* shows that physiological carriers of SH, such as ergothioneine and pantetheine, have no protective action at all. The protective action of a substance can therefore not be attributed to the presence of an —SH

### CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

group. In the molecule of cysteamine, the substitution of one or more of the hydrogen atoms combined with N or S diminishes or abolishes the protective action. The mono-, bi- or tri-acetyl derivatives have little activity, the sulphur containing methyl derivative is inactive, but the presence of a pantothenyl radical on the nitrogen also destroys the protective action. In cysteine, which is much less active than cysteamine, —COOH completely alters the physico-chemical properties of the molecule and makes the —NH<sub>2</sub> group less available. If the  $\beta$ -carbon of cysteine is methylated the molecule loses all its protective activity, though its —SH group remains intact<sup>57</sup>. Thioglycolic acid<sup>50</sup> and  $\beta$ -mercaptopropionic acid<sup>57</sup> have no protective action against radiations. We have fully proved that the —NH<sub>2</sub> group in the cysteamine molecule is at least as important as the —SH group<sup>2, 14, 50</sup>. Several aromatic amines without sulphur are excellent protectors against radiations (e.g. noradrenaline, tryptamine and 5-hydroxytryptamine<sup>14, 43</sup>.

Sulphur often seems to have a protective effect without being in the reduced state. Molecular colloidal sulphur (*i.e.* S=S) protects slightly *in vivo*<sup>29</sup> and well *in vitro*<sup>134</sup>, though in many reactions it plays the part of a hydrogen acceptor. For example a method of estimating free —SH groups using colloidal sulphur has been suggested<sup>51</sup>; the H<sub>2</sub>S formed is determined:



Thiourea\*, sodium ethane dithiophosphate and thiosulphate protect mice<sup>49-52</sup>, but large doses of thiouracil are needed<sup>2, 53, 55, 56</sup>. Thiouracil, which has a free —SH, has no protective action against radiations<sup>55</sup>; it hardly seems logical to attribute the effect of thiourea to its 'potential' —SH group<sup>54</sup>.

### CHEMICAL PROTECTION AND TISSUE ANOXIA

The intensity and universality of the oxygen effect have so much impressed radiologists that several of them, rightly or wrongly, attribute the radio-protective action to oxygen deprivation. We think this is possible in certain cases, but there are many examples where the idea of oxygen privation must be rejected.

HOLLAENDER and his collaborators<sup>6</sup> think that the protective action of a large number of substances which can be metabolized by *Escherichia coli* is due to the fact that, on entering the cells, they need

\* These facts have been discussed in their relation to thyroid function because thiourea, thiouracil and propyl-thiouracil are very active antithyroid substances. Animals with hyperthyroidism are more radiosensitive than normal animals, but those with hypothyroidism are not more resistant<sup>53, 54</sup>.

### CHEMICAL PROTECTION AND TISSUE ANOXIA

oxygen in order to be metabolized, and therefore lower the intracellular oxygen tension. Alcohols, glucose, amino-acids and the carboxylic acids of the cycle of Krebs are mentioned among these substances.  $\beta$ -Alanine, which is not utilized by *E. coli*, does not protect this organism, whereas  $\alpha$ -alanine is metabolized, and does protect it. If the catabolism of the carboxylic acids and alcohols is inhibited by cyanide, the protective action of these substances is diminished. Cyanide slightly diminishes the effect of cysteine, but not that of BAL or hydrosulphite (*Table V*).

*Table V. Relation between Inhibition of Respiration and Loss of Protection in Escherichia coli, according to HOLLAENDER and STAPLETON<sup>6</sup>*

Protective substance	D.R.F.* of protective agent only	D.R.F.* of protective agent in the presence of cyanide†	Per cent Loss of protection with cyanide	Per cent Inhibition of respiration with cyanide
Formate	3.0	1.0	100	70 to 85
Succinate	2.4 to 2.6	1.2	92	70 to 85
Pyruvate	3.0	1.1	62	20
Sérine	2.5	1.2	92	50
Cysteine	3.2	1.5	50	0
BAL	4.0	4.0	0	0
Ethanol	2.5 to 3.0	1.5	50	0
Sodium hydrosulphite.	4.0	4.0	0	0

\* D.R.F.=dose reduction factor= ratio of dose of x-rays needed to inactivate a given proportion of cells in the presence of a protector, to that needed in its absence.

† Concentration of cyanide, 0.002M.

HOLLAENDER, STAPLETON and BURNETT<sup>11,2</sup> have also suggested that giving carboxylic acids 'may supply some essential intermediate or overcome a block in the carboxylic acid cycle brought about by x-rays'. But, in that case, carboxylic acids should be equally effective *after* irradiation. We also considered this possibility, but our experiments on mice all gave negative results<sup>2</sup>.

TROWELL<sup>10,7</sup> found that lymph glands of rats, cultivated *in vitro*, are protected by lactate but not by glucose or by rat serum. It can be concluded that mammalian blood plasma has no protective action.

HOLLAENDER and STAPLETON<sup>6</sup> admit that the protection of *E. coli* by BAL, cysteine and sulphhydryl substances cannot be explained completely by oxygen privation, and that two possibilities must be considered: (i) competition with radiosensitive biological molecules for the free oxidizing radicals, and (ii) the replacement of a metabolite which is destroyed by radiations.

### CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

Investigations in mammals lead to the same conclusions. It is true that, in dogs, large doses of cysteine injected intravenously cause desaturation of oxygen in mixed venous blood from the right heart for about 15 minutes, and sometimes greatly lower the cardiac output, but in doses giving equal protection, cysteamine does not cause any anoxia<sup>92, 101</sup>.

The fact that cysteine is inactive, or less active, in the absence of oxygen in certain experiments<sup>102</sup> cannot lead to the conclusion that cysteine merely diminishes the intracellular oxygen tension. In the absence of oxygen the free radicals formed during irradiation with x- or  $\gamma$ -rays are not the same (no HO<sub>2</sub>), and we suppose that cysteine competes for these particular radicals (see p. 314). DEVIK<sup>103</sup>, studying the mortality of mice and the frequency of chromosome changes in the bone-marrow cells, found additive protection for hypoxia and sub-optimal doses of neutralized cysteine\*, injected intravenously; moreover the protective effect of d-cysteine (an isomer which is not natural and not utilized) is the same as that of L-cysteine, which suggests that the mechanism of protection is radiochemical rather than biochemical. This conclusion agrees perfectly with the elegant experiments of FORSSBERG<sup>153</sup> and NYBOM<sup>105</sup> on the roots of *Allium cepa* (see Figure 13). Glutathione<sup>108</sup> also decreases chromosome breakage in the root cells of *Tradescantia paludosa* irradiated with  $\gamma$ -rays from <sup>60</sup>Co. But cysteine had practically no effect in FORSSBERG and NYBOM's experiments<sup>105</sup> if the roots of *A. cepa* were irradiated with  $\alpha$ -rays, whose ionization is dense. Similarly, the protective effect of cysteine in mice is only half as great against neutrons as against x-rays<sup>109</sup>. PATT and his colleagues<sup>157</sup> have shown repeatedly that the protection of mice by cysteine is general and of the same order of magnitude whether the criterion used is mortality after 30 days, leucopaenia on the third day or rate of loss of weight after the third day. This observation suggests that the protector intervenes at a stage in the chain of the events between the absorption of the energy and the appearance of the biological lesion. All these results one would expect if the main action of cysteine is to compete for free oxidizing radicals (see p. 312). PATT *et al.*<sup>34, 109, 157</sup> on the other hand stress the cellular aspects of the problem since the amount of protection is not a unique function of the cysteine concentration and also since cysteine can protect isolated epidermal thymocytes if given immediately *after* irradiation. According to Patt cysteine decreases the oxygen tension in the interior of the cell.

---

\* Cysteine hydrochloride which has not been neutralized seems to be inactive (Devik<sup>104</sup>).

### CHEMICAL PROTECTION AND TISSUE ANOXIA

Other authors also attempt to interpret the protective action of certain vasoconstrictor amines as a result of tissue anoxia. This idea must be abandoned for the following reasons, which support a radiochemical interpretation (competition): (i) tryptamine, which is not a vasoconstrictor, is as good a protector of mice as 5-hydroxytryptamine (=serotonin), which is a vasoconstrictor<sup>14,43</sup>;

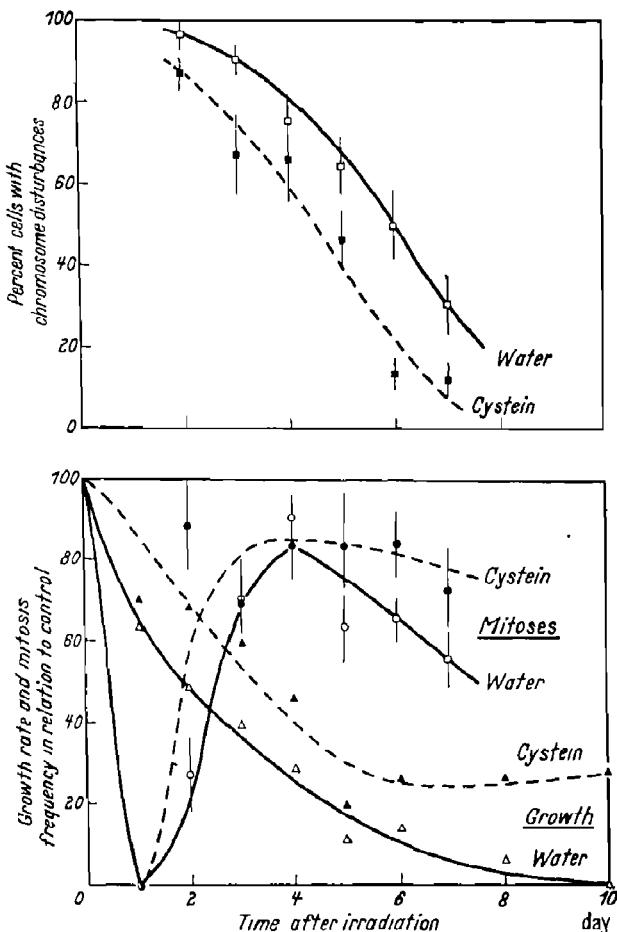


Figure 13. Chromosome disturbances, mitotic frequency and growth rate in *Allium cepa* after x-irradiation (740 r) in water and cysteine soln<sup>105</sup>

(ii) noradrenaline, and especially adrenaline, are vasoconstrictors only in certain parts of the body, e.g. the skin; they dilate many vessels, and increase oxygen consumption and the cardiac output;  
 (iii) all the aromatic amines, and especially tryptamine and its hydroxyl derivative are good protectors of polymethacrylate *in vitro*<sup>2</sup> (see Table IV).

### CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

Similarly, PATT<sup>4</sup> regards the liberation of histamine as the cause of the protective effect of cystamine ( $-\text{S}-\text{S}-$ ), the histamine causing a fall in oxygen tension through hypotension. This explanation is improbable, for (i) cystamine is a much more powerful and reproducible protector than histamine<sup>14, 43</sup>, and (ii) cystamine retains its activity when given by mouth, when the intervention of histamine is eliminated<sup>58</sup>.

WOLFF<sup>156</sup> has found what may prove to be an example of direct cellular protection (*i.e.* similar to that found with unicellular organisms) in the case of birds. After exposing specific parts of bird embryos to x-rays, monsters (*e.g.* cyclopia, anophthalmia) are regularly produced during the subsequent development. If the embryo has been previously injected with cysteamine the number of monsters which appears is greatly reduced.

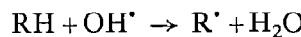
BACQ, ALEXANDER and their collaborators<sup>2</sup> have found a large number of substances whose protective action can hardly be explained by their direct or indirect production of anoxia. It is becoming more and more evident that the oxygen effect does not explain all the facts<sup>6, 99</sup>, and that competition for free radicals is probably the more general mechanism<sup>2</sup>.

### PHYSICOCHEMICAL MECHANISM OF PROTECTION

From the foregoing the most reasonable postulate seems to be that the protective agents interfere with one of the primary chemical reactions induced by x-rays and the following list represents the different mechanisms by which such an interference could take place.

(i) The protector may compete with vital cell constituents, which are inactivated by reaction, for the free radicals or other reactive substances produced in the body fluid. The classical work of DALE<sup>163, 164</sup> on the inactivation of enzymes by x-rays in dilute solutions demonstrated a protective mechanism of this type.

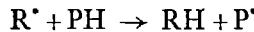
(ii) The reactive chemical entities formed in water may react with a vital macromolecule (*e.g.* RH) to give a free radical ( $\text{R}^{\cdot}$ ), *e.g.*



This radical may then undergo further reactions leading to loss of biological activity. In view of the enhancing effect which oxygen exercises in many forms of radiation damage (*cf.* Chapter 8) the reaction  $\text{R}^{\cdot} + \text{O}_2 \rightarrow \text{ROO}^{\cdot}$  may take place to give a peroxy-radical which is well known to be very unstable. The added chemical

### PHYSICOCHEMICAL MECHANISM OF PROTECTION

(PH) could then function by combining with either R<sup>•</sup> or ROO<sup>•</sup> to give a stable product which retains activity, or by acting as a transfer agent convert the free radical back to its original form, e.g.,



For an example of this type of protection *in vitro* see p. 131.

(iii) The added chemical may combine loosely with a receptor group (e.g. the prosthetic group of an enzyme) and thereby render it non-reactive towards free radicals\*. An interesting example of this type of action was demonstrated by DOHERTY<sup>165</sup> for chymotrypsin which could be protected against  $\gamma$ -rays by the addition of its substrate.

(iv) If the biologically important effect is direct (*i.e.* is brought about by the ionization of the biological material itself) it would seem that no protection is possible. However, it is known that organic materials in the solid or liquid state do not, in general, decompose immediately, but do so only when they can rearrange in such a way as to give two relatively stable products which do not recombine. The energy may thus be stored in a macromolecule for some time before decomposition occurs and in this interval it can be transferred to another molecule. Such energy transfer processes are well known in gas reactions and were recently demonstrated by ALEXANDER and CHARLESBY (see Chapter 4) with organic polymers.

In a biological system it is clearly not possible to determine by which of these processes the protective agent functioned since the primary changes produced by the ionizing radiations cannot be detected. A possible approach to this problem is to find an *in vitro* system in which the same protectors are active and to deduce the biological mechanism by analogy and this method has been followed by the present authors. As shown in Chapter 4, polymethacrylic acid in dilute aqueous solution is readily decomposed on irradiation with x-rays<sup>100, 167</sup>, and this reaction can be prevented

\* BACQ and HERVE<sup>14, 18</sup> have suggested the possibility that the protector, by combining with a radiosensitive enzyme, produces a radioresistant complex. After irradiation this complex slowly dissociates, the protector being eliminated or metabolized (for instance CN<sup>-</sup> becomes SCN<sup>-</sup> in the liver), and the enzyme becoming active again. This idea now seems to us superfluous, since the CN<sup>-</sup> ion has proved a good protector for polymers *in vitro*. It remains to be explained why animals resistant to cyanide are also resistant to radiations, and why, in a series of related species (Infusoria), sensitivity to cyanides and sensitivity to radiations run parallel<sup>32</sup>. Until the contrary is proved, it is logical to suppose that enzyme systems which enable animals resistant to cyanides to utilize oxygen even in the presence of cyanides are also systems resistant to radiations.

### CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

by the addition of protective agents. ALEXANDER<sup>166</sup> found that the same type of substances were active in the polymer system as in the mouse. Another point of similarity is that the degradation of the polymer requires the presence of dissolved oxygen.

A very extensive investigation<sup>2</sup> was undertaken by the present authors and almost 100 compounds were examined in the two systems. The agreement was truly remarkable (see *Tables III* and *IV*) for more than 80 per cent of the substances examined which included all the types discussed in the preceding pages. The behaviour of the substances ran completely parallel in the polymer and in the mouse. More than half of the discrepancies could be readily understood on metabolic grounds (*e.g.* cyanide and malononitrile are active in the mouse while only the former protects the polymer; but we know that malononitrile is converted to cyanide extremely rapidly *in vivo*), and only about six genuine exceptions remain. This parallelism is clearly not fortuitous and as a working hypothesis we can accept the view that the fundamental mechanism of protection is the same in the two systems. The degradation of the polymer was shown<sup>166-168</sup> to be brought about by HO<sub>2</sub> radicals formed in water (see p. 104) and the added substances could protect either by competing with the polymer for this radical or by combining with the unstable polymer radical before this has decomposed (*i.e.* mechanism (ii), p. 312).

This latter process is known as a transfer reaction and many instances of it are known, particularly in polymerization. Amongst the most effective transfer agents are SH compounds, and to a lesser extent amines. All substances having a reactive hydrogen atom would be expected to undergo a reaction of this type more or less readily. There is, however, strong evidence against the view that a transfer reaction is involved in protection. Firstly, only undisassociated amines can act as transfer agents, ammonium salts are quite inactive, yet some of the most effective amines have pK values higher than 10 and will be wholly in the cationic form at pH7. Moreover, it has been shown<sup>2</sup> in the polymer system that the protective action of amines is independent of pH and that aniline, for example, is equally active ionized as un-ionized. Secondly, thiourea derivatives, tryptamine hydrochloride and other good protecting substances do not act as transfer agents in polymerization reactions<sup>168</sup>. Thirdly, all SH compounds are outstanding transfer agents but only some are good protective agents (see p. 294). While these arguments do not rule out the possibility that a few isolated substances (*e.g.* iodine atoms produced photochemically) may protect by reacting with the unstable intermediate of polymethacrylic acid

#### PHYSICOCHEMICAL MECHANISM OF PROTECTION

the majority are believed to function by competing for HO<sub>2</sub> radicals.

Before any deductions concerning the role of the HO<sub>2</sub> radicals in the biological processes leading to death can be made by analogy with the *in vitro* results, it is necessary to establish that the protectors cannot compete equally for OH as for HO<sub>2</sub> radicals. To test this point the effect of protector on the *polymerization* of aqueous solutions of methacrylic acid by x-rays *in the absence of oxygen* was examined. Added substances can reduce the quantity of polymer formed by a given dose of x-rays either by competitively removing some of the initiating OH radicals or by reacting with the growing chain and thereby terminating it. By making reasonable assumptions concerning the mechanism of chain termination, these two reactions can be differentiated by measuring the molecular weight of the resulting polymer<sup>168</sup>. Thiourea and tryptamine, two most effective chemicals found for protecting the polymer against degradation, do not even at high concentrations influence the amount of x-ray catalysed polymerization, or the molecular weight of the resultant product. From this we conclude that they do not compete successfully with the monomer for OH radicals. On the other hand, other fairly active protectors such as hydroquinone decrease the amount of polymer formed, and there is no parallelism between inhibition of polymerization and protective action.

*Summary*—We therefore conclude that HO<sub>2</sub> radicals play an important role in those biological effects in which the protective agents studied by us are active and that these substances function by competitively removing these radicals whose general chemical reactivity is discussed on p. 104. This hypothesis also explains why the protective action of anoxia and that of the chemical substances run to some extent parallel and why cysteine fails to protect onion roots under anoxic conditions<sup>105</sup> since oxygen is necessary for the formation of HO<sub>2</sub> radicals. The finding that the protective agents are less active against radiation of high specific ionization is also in agreement with the competition mechanism since these substances would be expected to be less effective in removing radicals when these are formed in high local concentrations as occurs in the tracks of protons and  $\alpha$ -particles.

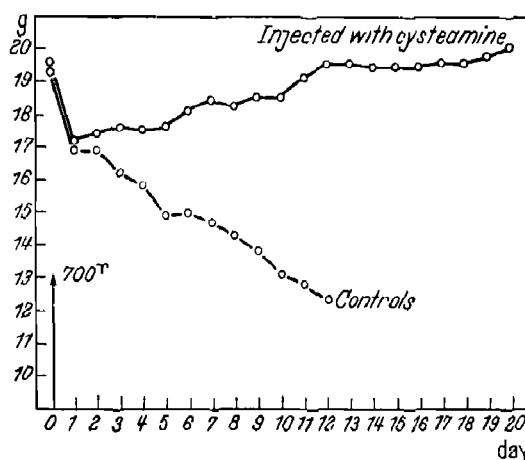
#### THE BEHAVIOUR OF PROTECTED ORGANISMS

A physiological, biochemical and cytological study of irradiated mammals is interesting from several points of view: the mode of action of protectors, the causes of survival, the behaviour of radio-sensitive

## CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

tissues, and changes in the mutagenic effects of radiations etc. Indeed, rodents 'protected' by cyanide, cysteine or mercaptoethylamine against a normally lethal dose of radiation, are, as it were, new beings, which arouse great interest (see 3-5, 7, etc.).

In spite of the great variety of protectors and animals used, one fact has been observed by most investigators during the days immediately following irradiation, namely that the behaviour of protected animals is, at first sight, practically the same as that of controls<sup>4, 5, 16, 48, 69, 114-117</sup>. The loss of weight, in other words the anorexia, is similar, although with cysteamine the protection of mice amounts to 100 per cent (*Figure 14 and 15*)\*. The leucopenia is



*Figure 14.* Mean weight of two series of irradiated mice: 10 controls (700 r) and 10 animals irradiated after intraperitoneal injection of 3 mg mercaptoethylamine. The weight drop is the same during the first two days; the chemically protected animals recover rapidly while the controls go on losing weight until death<sup>63</sup>

about the same for 4 to 8 days in animals protected by cysteamine as in controls (*Figures 15a and b*). The initial tissue destruction in the thymus, intestine and haematopoietic organs is the same, as far as can be seen<sup>48, 114, 118</sup>, and the loss of weight of the testicles is the same<sup>48</sup>. However, most authors agree† in saying that the regenerative processes, especially in the haematopoietic organs, occur earlier

\* LAMERTON<sup>93</sup> observed less loss of weight in rats which had received a large quantity of cysteamine and a dose of radiation which did not kill controls. The same applies to rats protected by glutathione and exposed to 800 r<sup>16</sup>.

† LORENZ<sup>117</sup> is about the only author who has not observed this earlier and more active regeneration in rats protected by cysteine.

THE BEHAVIOUR OF PROTECTED ORGANISMS

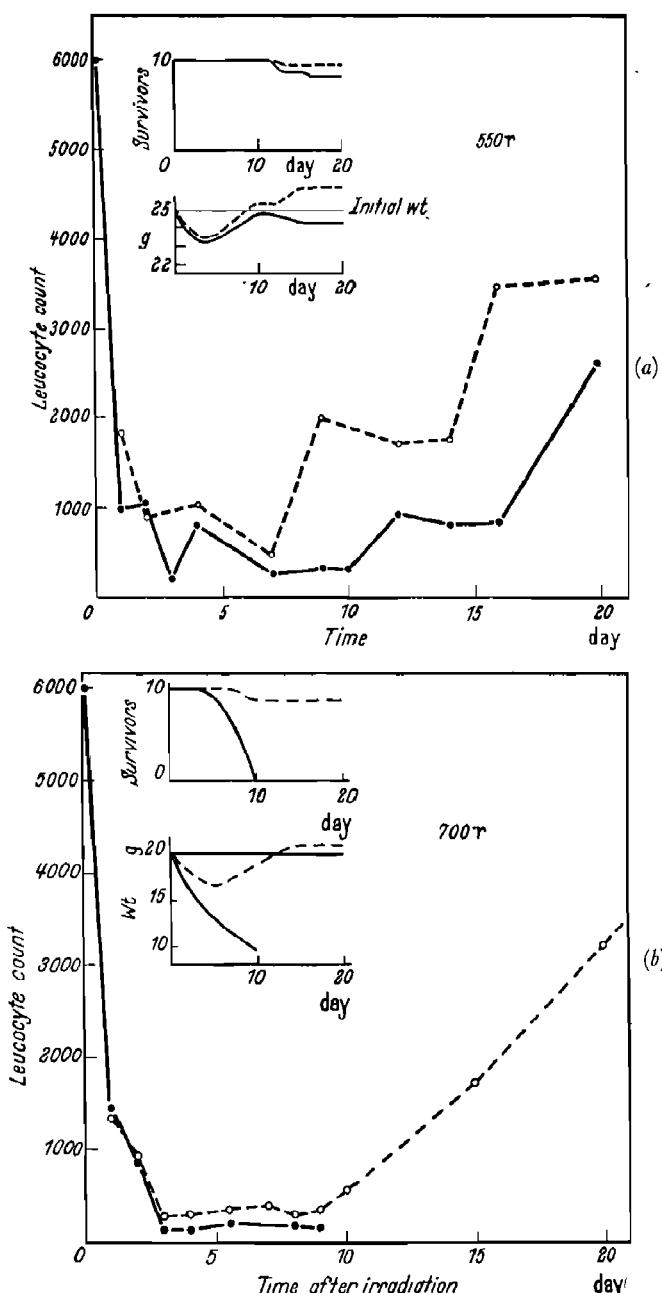


Figure 15. Leucocyte counts in peripheral blood of irradiated control mice, and mice irradiated with x-rays under protection of mercaptoethylamine. (a) 550 r of x-rays which is 10 per cent lethal for controls. Two small graphs indicate survival and variation in weight with 550 r in the particular strain of mice. The leucopenia is nearly the same in first 6 days in the two groups of animals, but the protected mice regenerate their white cells earlier<sup>63</sup>. (b) 700 r from which all control mice die with very low leucocyte count. Animals protected by cysteamine have slightly higher white cell count in first 9 to 10 days although 9 out of 10 survived<sup>63</sup>

----- irradiated after injection with cysteamine  
 ——— irradiated controls without cysteamine

### CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

and are more intense in protected animals than in controls<sup>48, 63, 114, 115, 119</sup>. The spleen of rats and mice lends itself well to investigations of this kind. According to GROS, MANDEL and RODESH<sup>119</sup> the nucleic acid content (DNA and RNA) of the spleen of rats protected by cysteamine falls 24 and 48 hours after irradiation, as in controls, but on the fourth day the spleen of the protected animals contains 70 per cent more nucleic acid. There is a second peak about the fourteenth day, and by the twentieth day there is no longer any difference. BETZ<sup>114-116</sup> emphasizes the fact that, 6 to 7 days after irradiation, the adrenal cortex is less affected, *i.e.* less depleted, in mice protected by cyanide than in controls, and less lipid is accumulated by the liver in protected animals. Hence the supposition that the cortical hormones play a part in the regeneration of the haematopoietic tissues<sup>115, 142</sup>. The effect of cysteamine on the radiation response of the suprarenals<sup>143</sup> has already been described on p. 275.

CRONKITE's interpretation<sup>48</sup> has attracted the attention of all who have studied these facts (see especially<sup>69</sup>): 'It appears that glutathione by an unknown manner protects the mechanisms which accelerate the regeneration of the hemopoietic tissues, but does not prevent the cellular destruction.' This sums up satisfactorily most observations made on animals protected with cyanide, cysteine and glutathione, and a fair proportion of those protected with cysteamine (see also LACASSAGNE<sup>144</sup>). For example, regeneration of the seminal series is also much accelerated in mice if cysteamine as benzoate or salicylate is ingested 30 to 70 minutes before total irradiation with 600 r<sup>131</sup>. Certain biological effects of x-rays do not seem to be diminished by cysteamine. For instance, if injected *before* irradiation, cysteamine protects neither mice nor *Drosophila* from the mutagenic effect of x-rays<sup>127, 128\*</sup>. This is important, for it is generally accepted that the mutagenic and carcinogenic effects are parallel. But HERVE and NEUKOMM<sup>129</sup> have shown that mercaptoethylamine, provided it is injected in large doses (200 mg/kg) slightly reduces the effect of x-rays (3000 r) applied locally to Gaspari's mammary adenocarcinoma transplanted into mice.† DESAIVE<sup>130</sup> has determined the sterilizing dose for female

\* According to ELDJARN<sup>162</sup> this might be due, at least in the mouse, to the fact that the concentration of cysteamine in the testis reaches only one-tenth of the corresponding value in the bone-marrow.

† There is, however, no danger of diminishing the effect of the rays on the tumour if cystamine or cysteamine is given *after* irradiation to prevent the general symptoms of radiation sickness (nausea, vomiting, diarrhoea, headache, vertigo, asthenia etc.) (see p. 345).

THE BEHAVIOUR OF PROTECTED ORGANISMS

rabbits with and without protection by cysteamine (25 mg/kg intraperitoneally) :

		Single dose	Fractioned dose
<i>Normal female rabbit</i>	.	2500	2000
<i>Female rabbit injected with cysteamine</i>	:	3000	2750

This gives a protective factor of 1.2 to 1.4, which is the same as that which we found for the tissues which produce lymphocytes in mice<sup>63</sup>, and lower than the factor of 1.8 to 2 obtained when the effect tested is the mortality of mice<sup>69</sup>.

But even if Cronkite's general interpretation is accepted, the sources of this hypothetical regenerative factor must be less affected in protected animals than in controls. A quantitative histological study is therefore of the greatest interest, because the organs partly protected against the *primary* effects of x-rays must be regarded as the possible sources of the regenerative factor. GEREBTZOFF and BACQ<sup>118</sup> have carefully compared the liver, spleen, intestinal mucosa and thymus of mice irradiated with 700 r, some with and some without the protection of 150 mg/kg cysteamine. The animals were killed 6 hours or 4 days after irradiation. It is assumed that what is seen after 6 hours is mainly the degenerative processes which are the *primary* effects of x-rays, whereas after 4 days the intensity of *regeneration* is chiefly noticed. By choosing appropriate objective criteria in each case, and after statistical analysis of the results it is found (see *Table VI*) that :

*Table VI. Histological Findings after Irradiation with x-rays (700 r) in C57 Mice with and without Protection by Intraperitoneal Injection of 150 mg/kg cysteamine<sup>118</sup>*

	<i>Controls killed after 6 h</i>	<i>Protected mice after 6 h</i>		<i>Controls killed after 4 days</i>	<i>Protected mice killed after 4 days</i>
<i>Spleen</i>	0.063-0.221 (0.151)	0.290-0.471 (0.371)		<i>Pyknotic nuclei present</i>	<i>Pyknotic nuclei very rare</i>
<i>Degree of pyknosis*</i>					
<i>Thymus</i>	0.217-0.287 (0.242)	0.229-0.300 (0.268)	<i>No. of mitoses per 10 fields</i>	40-54 (48)	45-66 (60)
<i>Degree of pyknosis†</i>					
<i>Intestine</i>	40-55 <i>No. of pyknotic nuclei per 10 fields</i>	29-61 (45)	<i>No. of mitoses per 10 fields</i>	51-71 (61)	80-93 (83)

Figures in brackets are averages.

\* Ratio of intact surface to total surface of lymphoid follicles.

† Ratio of transverse diameter of intact regions to transverse diameter of lobe of thymus.

#### CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

(i) The degree of *degeneration* is the same in the thymus and in the intestines; (ii) regeneration is distinctly greater in the intestines of protected animals than in controls. In the thymus the difference between the protected animals and the controls is not quite significant ( $SS = 2.66$ , whereas it should be 3); (iii) the degree of *degeneration* of the spleen is less in the protected animals, which suggests that, in this organ, cysteamine decreases the *primary* effect of x-rays.

The liver behaves in the same way as the spleen, that is, the liver tissue is less affected after 6 hours in protected animals than in controls (*Figures 16 and 17*), in particular, the cytoplasm is less permeated with water. Certain organs (the liver and spleen in C57 mice), therefore, are truly protected, whereas in other organs (*e.g.* the intestines) the protector merely accelerates regeneration. In starving rats, irradiation increases the glycogen content of the liver. This increase, which reaches its maximum in 32 hours, does not take place if the animal is irradiated when protected by cysteamine<sup>132</sup>. DEVIK<sup>103</sup> has observed that neutralized cysteine, injected intravenously, diminishes the frequency of chromosome changes in the bone-marrow; here again, the effect is a diminution of the *primary* effect of radiations. That such a primary protective action at the cellular level should be visible in liver and spleen is consistent with our hypothesis that the main action of cysteamine is to compete with radiosensitive cellular constituents for the  $\text{HO}_2$  radicals (see p. 314). The fact that certain tissues do not show this early protection-effect may be due either to the fact that cysteamine is not concentrated by these tissues or that the dose for maximal histological damage is low (350 r or less) and that consequently no difference can be seen by microscopical examination if the action of free radicals during irradiation has been reduced by half. These facts must be considered in relation to the many observations which show that homogenates of spleen and bone-marrow, injected after irradiation, accelerate the regeneration of the haematopoietic organs (see Chapter 16) and are quite consistent with Maisin's observations<sup>158</sup> that the protective action of cysteamine is additive to that obtained by local lead shielding.

The best interpretation of the survival of irradiated rodents protected by cysteamine and cysteine (and perhaps by other protectors) therefore seems to be that the protector diminishes the cytolytic, destructive, effects of ionizing radiations on the spleen and bone-marrow, which are consequently fit, after 24 to 48 hours, not only to regenerate more actively, but also to supply the body with the regenerative factor or factors needed by certain tissues. Indeed, it

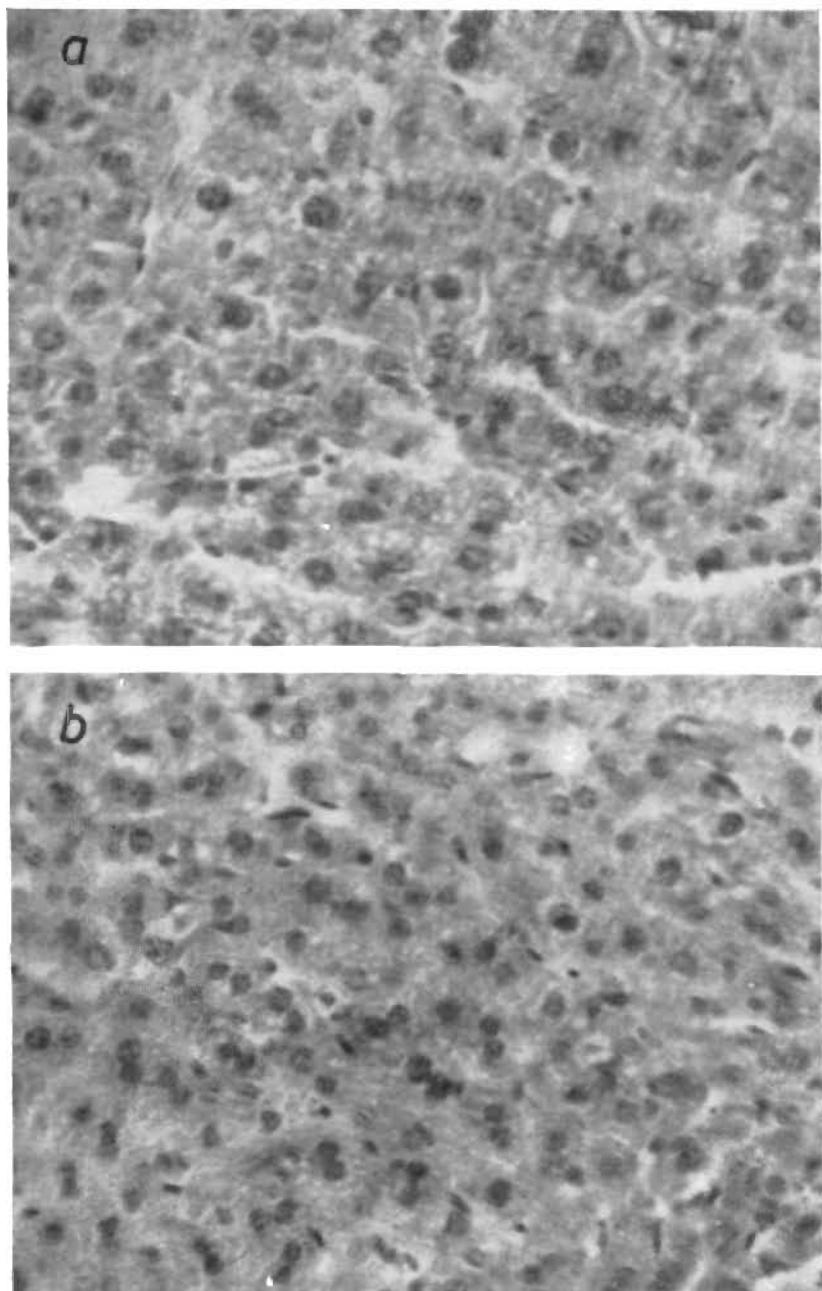


Figure 16 (a) Liver of mouse 6 h after 700 r whole-body irradiation with x-rays.  
(b) Liver of mouse similarly irradiated, but after injection of 3 mg cysteamine/20 g mouse.  
Fixation formaldehyde, picric acid (Bouin); hemalum eosine. Predominance of spongy aspect  
of hepatic tissue in the non-protected animal

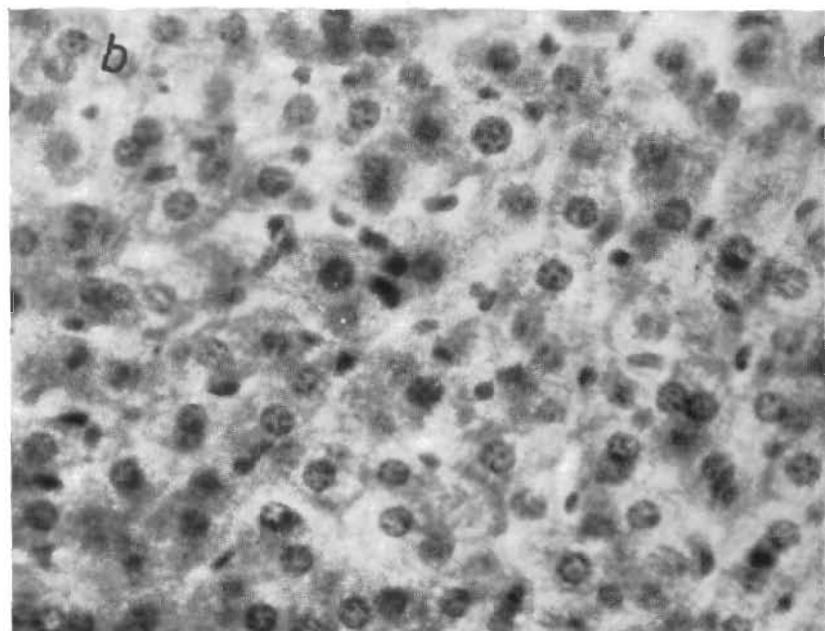
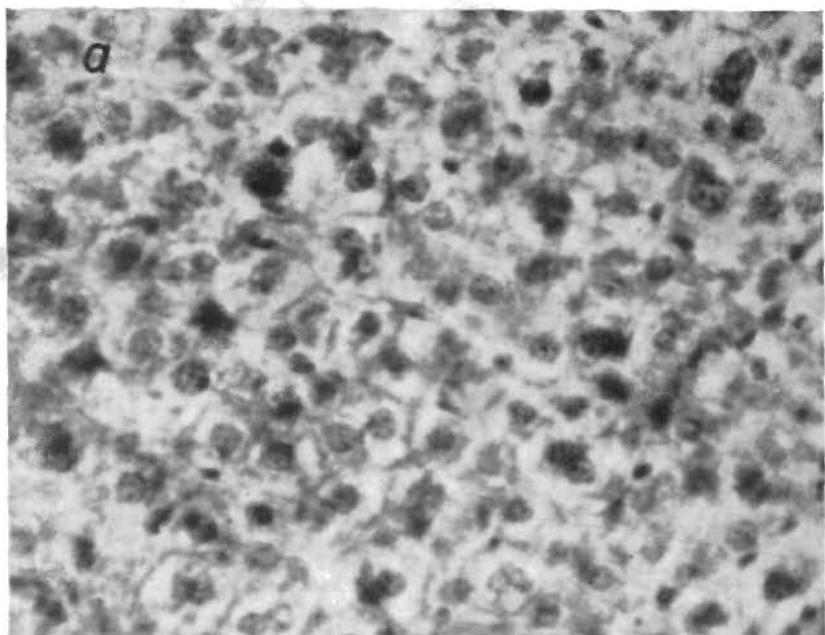


Figure 17 (a) Liver of mouse 4 days after 700 r whole-body irradiation with x-rays. (b) Liver of mouse 4 days after similar irradiation, but protected by cysteamine. Fixation and staining as in Figure 16. The very sponge-like aspect of hepatic cells of the unprotected mouse contrasts with the practically normal structure of the liver of the protected animal

#### THE BEHAVIOUR OF PROTECTED ORGANISMS

is probable that, in certain circumstances, these factors (see p. 341) diffuse from the cell nuclei where they seem to concentrate.

It remains to be understood why some tissues act differently from others when they are irradiated under the protection of cysteamine. We think that the main reason is that this substance is concentrated by certain tissues and not by others. It has been proved by the investigations, many of them not yet published, of three groups (Oslo, Liège and Louvain), using molecules labelled with  $^{35}\text{S}$ , that cysteamine becomes concentrated in the bone-marrow and kidneys. In the last-named organ the concentration is only due to excretion.

#### PRACTICAL APPLICATIONS

Armed forces and Civil Defence personnel (even, in certain cases, the civilian population) can safely ensure the greatest possible *chemical protection* by ingesting 500 to 800 mg of cystamine 2HCl 15 to 20 minutes before entering a dangerously radioactive area. The protection gradually diminishes, because this amine is metabolized and excreted in the urine fairly quickly, but after 4 to 5 hours the protective effect is still considerable\*<sup>58</sup>. If 15 to 30 minutes is too long to wait, a slow injection of 200 to 400 mg cysteamine, which has a more rapid but less lasting effect (about 1 hour), may be given<sup>14</sup>.

Other protectors which may be used in time of war will probably be discovered in the next few years, as we learn more about their action. However, the fact that cysteamine and cystamine are not foreign to the body makes them particularly interesting.

In peace-time medicine, radiotherapists who use one of these molecules (preferably cystamine by mouth) *after* irradiation can be sure that they are not diminishing the therapeutic effects of x- or  $\gamma$ -rays, as the irradiation does not take place when the protector is present in the tissues. A number of experiments (see especially<sup>82</sup>) have shown that in man, even after prolonged treatment with large doses of mercaptoethylamine, the response of pathological tissues to ionizing rays remains normal. These protectors do not induce radioresistance. This fact agrees perfectly with the theory of competition for free radicals ( $\text{HO}_2$ ) which we are putting forward<sup>2</sup>.

If there was a protector against radiations which did not penetrate into cancer cells (or at least certain cancer cells), the possibility of irradiating these cells with much larger doses or more widely might be considered, as the rest of the body would be protected by this substance. This would, of course, need prolonged

---

\* Research for this purpose has been undertaken at the request of the Belgian Government (Conseil Supérieur de la Sécurité Civile)

## CHEMICAL PROTECTION AGAINST X- AND $\gamma$ -RAYS

research, but we believe the chances of obtaining a useful result are good. In fact, the opposite phenomenon is already being studied: there are substances which increase radiosensitivity, and which accumulate in tumours.

### SUBSTANCES WHICH INTENSIFY THE EFFECTS OF X-RAYS

Mitchell and his collaborators have, since, 1946, systematically studied the substances which increase sensitivity to ionizing radiations, from the chemical, experimental and clinical points of view. An excellent account of these studies and a full bibliography are to be found in Mitchell's recent publications<sup>133-135</sup>.

The best known molecule, with which clinical tests have been carried out ever since 1946, is a phosphoric ester of a substance related to vitamin K, the tetrasodium salt of 2-methyl-1:4-naphtho-hydroquinone diphosphate (or Synkavit). The 2-3 dimethyl derivative of this compound seems still more interesting. A study of the relation between chemical composition and biological activity shows: (i) that in derivatives of Synkavit the presence of phosphates is necessary, and (ii) that the biological effects do not depend solely on reaction with —SH groups, though this reaction seems to play some part. Treatment with Synkavit *before* irradiation increases the mortality of rats after exposure of the whole body to x-rays. Experiments on rats with Walker's sarcoma have shown increased fluorescence in Wood's light of certain tissues and of actively growing parts of the tumour after the injection of certain derivatives of Synkavit. These derivatives are much more selectively concentrated in the tumour if they are injected intravenously. Both experimentally and clinically, the intravenous route of administration is the only one which has proved useful. Intramuscular injections have practically no effect on the behaviour of tumours irradiated with x-rays.

Mitchell has set out clearly how clinical tests should be carried out in this difficult domain in order to be valid. Experiments are still in progress. The only definite results so far have been obtained with inoperable bronchial carcinoma, in which, according to Mitchell's statistics, the average duration of life after the first irradiation is 4 months. If radiotherapy is combined with intravenous injections of Synkavit the duration of life is increased to 11 months; if intramuscular injections are given the period of survival is increased only to 6 months.

It may be concluded, therefore, that intravenous injections of this compound (Synkavit) have a slight beneficial influence on the results of radiotherapy.

## REFERENCES

## REFERENCES

- <sup>1</sup> BACQ, Z. M., *Experientia*, 1951, **7**, 11
- <sup>2</sup> ALEXANDER, P., BACQ, Z. M. et al., *Radiation Res.*, 1954, in press
- <sup>3</sup> PATT, H. M., *Annu. Rev. nucl. Sci.*, 1952, **1**, 495
- <sup>4</sup> PATT, H. M., *Physiol. Rev.*, 1953, **33**, 35
- <sup>5</sup> BRUES, A. M. and PATT, H. M., *Physiol. Rev.*, 1953, **33**, 85
- <sup>6</sup> HOLLAENDER, A. and STAPLETON, G. E., *ibid.*, 1953, **33**, 77
- <sup>7</sup> ORD, M. G. and STOCKEN, L. A., *ibid.*, 1953, **33**, 356
- <sup>8</sup> SELLE, W. A., MASON, G. D. and NEWMAN, R. H., *Univ. Calif. Atom. Energy Proj.*, UCLA-264, 1953
- <sup>9</sup> LACASSAGNE, A., *Fortschr. Roentgenstr.*, 1951, **75**, 98
- <sup>10</sup> LANGENDORFF, H., *Strahlentherapie*, 1952, **88**, 164
- <sup>11</sup> MACK, H. P. and FIGGE, F. H. J., *Anat. Rec.*, 1951, **111**, 547
- <sup>12</sup> SMITH, F. and SMITH, W. W., *Amer. J. Physiol.*, 1951, **165**, 662
- <sup>13</sup> PATERSON, E. and MATTHEWS, J. J., *Nature, Lond.*, 1951, **168**, 1126
- <sup>14</sup> BACQ, Z. M. and HERVE, A., *Bull. Acad. Méd. Belg.*, 1952, 6th series, **17**, 13
- <sup>15</sup> BONET-MAURY, P. and PATTI, F., *J. Radiol. Electrol.*, 1950, **31**, 286
- <sup>16</sup> CHAPMAN, W. H., SIPE, C. R., ELTZHOLTZ, D. C., CRONKITE, E. P. and CHAMBERS, F. W., *Radiology*, 1950, **55**, 865
- <sup>17</sup> HERVE, A. and BACQ, Z. M., *C.R. Soc. Biol.*, 1949, **143**, 881, 1158
- <sup>18</sup> BACQ, Z. M. and HERVE, A., *Brit. J. Radiol.*, 1951, **24**, 617
- <sup>19</sup> BACQ, Z. M., HERVE, A., LECOMTE, J. and FISCHER, P., *Science*, 1951, **111**, 356
- <sup>20</sup> HERVE, A., BACQ, Z. M. and BETZ, H., *J. Chim. phys.*, 1951, **48**, 256
- <sup>21</sup> BETZ, H. and HERVE, A., *C.R. Soc. Biol.*, 1950, **144**, 1015
- <sup>22</sup> MAZZANTI, L. and FRANCHI, M., *Arch. Ital. Sci. farm.*, 1952, (3) **2**, 261
- <sup>23</sup> SMITH, D. E., PATT, H. M. and TYREE, E. B., *Nucl. Sci. Abstr.*, 1951, **5**, 2032
- <sup>24</sup> DOWDY, A. H., BENNETT, L. R. and CHASTAIN, S. M., *Radiology*, 1950, **55**, 879
- <sup>25</sup> MEFFRED, R. B. Jr., and MATNEY, T. S., *Science*, 1952, **115**, 116
- <sup>26</sup> BOYLAND, E. and GALlico, E., *Brit. J. Cancer*, 1952, **6**, 160
- <sup>27</sup> BERGER, H., HAAS, F. L., WYSS, O. and STONE, W. S., *J. Bact.*, 1953, **65**, 538
- <sup>28</sup> PATT, H. M., TYREE, E. B., STRAUBE, R. L. and SMITH, D. E., *Science*, 1949, **110**, 213
- <sup>29</sup> PATT, H. M., SMITH, D. E., TYREE, E. B. and STRAUBE, R. L., *Proc. Soc. exp. Biol. Med.*, 1950, **73**, 18
- <sup>30</sup> BACQ, Z. M. and HERVE, A., unpublished observations, 1953
- <sup>31</sup> PHILPOT, J. St.-L., personal communication, 1952
- <sup>32</sup> BACQ, Z. M., MUGARD, H. and HERVE, A., *Acta Radiol.*, 1952, **38**, 489
- <sup>33</sup> NIZET, A., BACQ, Z. M. and HERVE, A., *Arch. int. Physiol.*, 1952, **60**, 448
- <sup>34</sup> PATT, H. M., BLACKFORD, M. E. and STRAUBE, R. L., *Proc. Soc. exp. Biol. Med.*, 1952, **80**, 92
- <sup>35</sup> MOLE, R. H., *Brit. J. Radiol.*, 1953, **26**, 234

CHEMICAL PROTECTION AGAINST X- AND  $\gamma$ -RAYS

- <sup>36</sup> PATT, H. M. and SWIFT, M. N., *Amer. J. Physiol.*, 1948, **155**, 388
- <sup>37</sup> CRABTREE, H. G. and CRAMER, W., *Proc. roy. Soc.*, 1933, **113B**, 238
- <sup>38</sup> MOTTRAM, J. C., *Brit. J. Radiol.*, 1935, **8**, 32
- <sup>39</sup> BACQ, Z. M. and HERVE, A., *Arch. int. Physiol.*, 1951, **59**, 348
- <sup>40</sup> D'AMATO, F. and GUSTAFSSON, A., *Hereditas*, 1948, **34**, 181
- <sup>41</sup> BACQ, Z. M. and HERVE, A., *Nature, Lond.*, 1951, **168**, 1126
- <sup>42</sup> BACQ, Z. M., HERVE, A. and FISCHER, P., *Bull. Acad. Méd. Belg.*, 1953, 6th series, **18**, 226
- <sup>43</sup> BACQ, Z. M. and HERVE, A., *J. suisse Med.*, 1952, **82**, 1018
- <sup>44</sup> COLE, L. J. and ELLIS, M. E., *Amer. J. Physiol.*, 1952, **170**, 724
- <sup>45</sup> PUCKETT, N., BELLACK, S. and KREBS, A. T., AMRL-77, 1952
- <sup>46</sup> HURSH, J. B., *Proc. Soc. exp. Biol. Med.*, 1952, **79**, 210
- <sup>47</sup> CHAPMAN, W. H. and CRONKITE, E. P., *ibid.*, 1950, **75**, 318
- <sup>48</sup> CRONKITE, E. P., BRECHER, G. and CHAPMAN, W. H., *ibid.*, 1951, **76**, 396
- <sup>49</sup> MOLE, R. H., *J. Chem. phys.*, 1951, **48**, 258
- <sup>50</sup> BACQ, Z. M., HERVE, A., LECOMTE, J., FISCHER, P., BLAVIER, J., DECHAMPS, G., LE BIHAN, H. and RAYET, P., *Arch. int. Physiol.*, 1951, **59**, 442
- <sup>51</sup> HEUSGHEN, C., Personal communications, 1953
- <sup>52</sup> MOLE, R. H., PHILPOT, J. ST. L. and HODGES, G. R. V., *Nature, Lond.*, 1950, **166**, 515
- <sup>53</sup> HALEY, T. J., MANN, S. and DOWDY, A. H., *Science*, 1951, **114**, 153
- <sup>54</sup> HALEY, T. J., MANN, S. and DOWDY, A. H., *J. Amer. pharm. Ass.*, 1952, **41**, 39
- <sup>55</sup> LIMPEROS, G., *Amer. J. Roentgenol.*, 1952, **67**, 810
- <sup>56</sup> LIMPEROS, G. and MOSHER, W. A., *Science*, 1950, **112**, 86
- <sup>57</sup> LORENZ, W., *Fortschr. Roentgenstr.*, Special Congress No., Wiesbaden, 1952
- <sup>58</sup> BACQ, Z. M., *Bull. Acad. Méd. Belg.*, 1953, 6th series, **18**, 426
- <sup>59</sup> ELDJARN, L., BADDILEY, J., NIZET, A. and VERLY, W., personal communications, 1952
- <sup>60</sup> ELDJARN, L., *J. biol. Chem.*, in press
- <sup>61</sup> VERLY, W., BACQ, Z. M., RAYET, P. and URBAIN, *Acta Bioch. Biophys.*, in press, 1954
- <sup>62</sup> VERLY, W. and BACQ, Z. M., unpublished observations, 1954
- <sup>63</sup> BACQ, Z. M., HERVE, A. and SCHERBER, F., *Arch. int. Pharmacol. Thér.*, 1953, **94**, 93
- <sup>64</sup> FISCHER, P. and GOUTIER-PIROTTE, M., *Arch. int. Physiol.*, 1954, **62**, 76
- <sup>65</sup> BACQ, Z. M., FISCHER, P. and PIROTTE, M., *ibid.*, 1952, **60**, 559
- <sup>66</sup> BACQ, Z. M., FISCHER, P. and PIROTTE, M., *ibid.*, 1952, **60**, 535
- <sup>67</sup> ELDJARN, L., MAISIN, J. et al., personal communications
- <sup>68</sup> LECOMTE, J., *Arch. int. Physiol.*, 1952, **60**, 179
- <sup>69</sup> BACQ, Z. M., DECJIAMPS, G., FISCHER, P., HERVE, A., LE BIHAN, H., LECOMTE, J., PIROTTE, M. and RAYET, P., *Science*, 1953, **117**, 633
- <sup>70</sup> PECZENIK, O., *Nature, Lond.*, 1953, **172**, 454
- <sup>71</sup> DEYSSON, G. and TRUHAUT, R., *C.R. Acad. Sci., Paris.*, 1953, **236**, 2329
- <sup>72</sup> DEYSSON, G. and TRUHAUT, R., *Bull. Soc. Chim. biol.*, 1953, **35**, 1019
- <sup>73</sup> KLUYSKENS, P., *C.R. Soc. Biol.*, 1953, **147**, 733

#### REFERENCES

- <sup>74</sup> KLUYSKENS, P., *Nature, Lond.*, 1953, **172**, 912
- <sup>75</sup> BACQ, Z. M. and HERVE, A., *7th int. Congr. Radiol.*, Copenhagen, 1953, p. 133
- <sup>76</sup> HERVE, A. and BACQ, Z. M., *J. Radiol. Electrol.*, 1952, **33**, 651
- <sup>77</sup> LECOMTE, J. and BOHRENSYAYN, CH., *C.R. Soc. Biol.*, 1953, **147**, 743
- <sup>78</sup> LECOMTE, J., VAN CAUWENBERGHE, H. and GOBLET, J., *ibid.*, 1953, **147**, 1121
- <sup>79</sup> VAN DE BERG, L. and LECOMTE, J., *Arch. int. Physiol.*, 1953, **61**, 240
- <sup>80</sup> LECOMTE, J., VAN CAUWENBERGHE, H., GOBLET, J. and VLIERS, M., *Ann. Endocr.*, 1953, **14**, 123
- <sup>81</sup> CHEVREMONT, S. and M., *C.R. Soc. Biol.*, 1953, **147**, 164; *C.R. Ass. Anat., Bordeaux*, 1953
- <sup>82</sup> BACQ, Z. M., BERNARD, J., RAMIOUL, H. and DELTOUR, G., *Bull. Acad. Méd. Belg.*, 1952, 6th series, **17**, 460
- <sup>83</sup> GENNES, L. de, DELTOUR, G., TOURNEUR, R. and LEPRAT, J., *Bull. Soc. Méd. Hôp., Paris*, 1953, p. 108
- <sup>84</sup> VAN CAUWENBERGHE, H., ROSKAM, J., HEUSGIEM, C., FISCHER, P., DELTOUR, G. and BACQ, Z. M., *Arch. int. Physiol.*, 1953, **61**, 124
- <sup>85</sup> BACQ, Z. M. and FISCHER, P., *Arch. int. Physiol.*, 1953, **61**, 417
- <sup>86</sup> FISCHER, P. and BACQ, Z. M., *Arch. int. Physiol.*, 1952, **60**, 541
- <sup>87</sup> LOISELEUR, J. and VELLEY, G., *C.R. Acad. Sci., Paris*, 1950, **231**, 182
- <sup>88</sup> GRAY, J. L., TEW, J. T. and JENSEN, H., *Proc. Soc. exp. Biol. Med.*, 1952, **80**, 604
- <sup>89</sup> RUGH, R. and WANG, S. C., *ibid.*, 1953, **83**, 411
- <sup>90</sup> GRAY, J. L., MOULDEN, E. J., TEW, J. T. and JENSEN, H., *ibid.*, 1952, **79**, 384
- <sup>91</sup> STRAUBE, R. L. and PATT, H. M., *ANL, Mon. Rep.*, Oct. 1953, p. 17
- <sup>92</sup> HOLLANDER, A., Aarhus Conference, July, 1953, see *Nucleonics*, Dec. 1953, **11**, No. 12, 18
- <sup>93</sup> LAMERTON, F., *Brit. J. Radiol.*, 1953, **26**, 510, 568
- <sup>94</sup> MAISIN, J. H., LAMBERT, G., MANDART, H. and MAISIN, H., *Nature, Lond.*, 1953, **171**, 971
- <sup>95</sup> BACQ, Z. M. and EULER, U. S. von, 'Symp.: Hormones protéiques et dérivées des protéines', *2nd Congr. int. Bioch., Paris*, 1952
- <sup>96</sup> LOISELEUR, J. and VELLEY, G., *C.R. Acad. Sci., Paris*, 1950, **231**, 529
- <sup>97</sup> STORER, J. B. and COON, J. M., *Proc. Soc. exp. Biol. Med.*, 1950, **74**, 202
- <sup>98</sup> COLE, L. J., BOND, V. P. and FISCHLER, M. C., *Science*, 1952, **115**, 644
- <sup>99</sup> COLE, L. J. and ELLIS, M. E., *Amer. J. Physiol.*, 1953, **175**, 429
- <sup>100</sup> ALEXANDER, P. and FOX, M., *Nature, Lond.*, 1952, **170**, 1022
- <sup>101</sup> CHARLIER, R., *Proc. Soc. exp. Biol. Med.*, 1954, **86**, 290
- <sup>102</sup> BLACKFORD, M. E. and PATT, H. M., *ANL, Quart. Rep.*, 1953, 4840
- <sup>103</sup> DEVIK, F., *Brit. J. Radiol.*, 1954, in press
- <sup>104</sup> DEVIK, F., *ibid.*, 1952, **25**, 481
- <sup>105</sup> FORSSBERG, A. and NYBOM, N., *Physiol. Plant.*, 1953, **6**, 78
- <sup>106</sup> KAHN, J. B. Jr., *Proc. Soc. exp. Biol. Med.*, 1951, **78**, 486
- <sup>107</sup> TROWELL, O. A., *Brit. J. Radiol.*, 1953, **26**, 302
- <sup>108</sup> MIKAELSEN, K., *Science*, 1952, **116**, 172

- 109 PATT, H. M., CLARK, J. W. and VOGEL, H., *Proc. Soc. exp. Biol. Med.*, 1953, **84**, 189  
 110 THOMPSON, T. L., MEFFRED, R. B. Jr. and Wyss, O., *J. Bact.*, 1951, **62**, 39  
 111 DEUEL, H. J. Jr., CHENG, A. L. S., KRYDER, G. D. and BINGEMANN, M. E., *Science*, 1953, **117**, 254  
 112 HOLLAENDER, A., STAPLETON, G. E. and BURNETT, W. T. Jr., *Isotopes in Biochemistry* (Ciba Conf.), Churchill, London, 1951, p. 96  
 113 PARR, W., O'NEILL, T. and KREBS, A., *Science*, 1953, **117**, 155  
 114 BETZ, H., *Acta Un. int. Cancer*, 1952, **7**, 814  
 115 BETZ, H., *C.R. Soc. Biol.*, 1950, **144**, 593, 1437  
 116 BETZ, H. and FRUHLING, L., *ibid.*, 1950, **144**, 1013  
 117 LORENZ, W., *Strahlentherapie*, 1952, **88**, 190  
 118 GEREBTZOFF, M. A. and BACQ, Z. M., *Experientia*, 1954, **10**, 341  
 119 GROS, C., MANDEL, P. and RODESCH, J., *C.R. Acad. Sci., Paris*, 1953, **236**, 2010  
 120 BURNETT, W. T., MORSE, M. L., BURKE, A. W. Jr. and HOLLAENDER, A., *J. Bact.*, 1952, **63**, 591  
 121 TREADWELL, A. de G., GARDNER, W. V. and LAWRENCE, J. H., *Endocrinology*, 1943, **32**, 161  
 122 KAPLAN, H. S. and BROWN, M. B., *Cancer Res.*, 1951, **11**, 706  
 123 KAPLAN, H. S., BROWN, M. B. and MARDER, S. N., *ibid.*, 1951, **11**, 262  
 124 BONET-MAURY, P. and PATTI, F., *J. Radiol. Electrol.*, 1953, **34**, 636  
 125 STRAUBE, R. L. and PATT, H. M., *Proc. Soc. exp. Biol. Med.*, 1953, **84**, 702  
 126 LAMBERT, S., *Arch. int. Physiol.*, 1954, **62**, 132  
 127 KAPLAN, W. D. and LYON, M. F., *Science*, 1953, **118**, 776  
 128 KAPLAN, W. D. and LYON, M. F., *ibid.*, 1953, **118**, 778  
 129 HERVE, A. and NEUKOMM, S., *Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955  
 130 DESAIVE, P., *Acta Radiol.*, 1954, in press  
 131 DESAIVE, P., BACQ, Z. M. and HERVE, A., *J. belge Radiol.*, 1953, **36**, 505  
 132 FISCHER, P., *Arch. int. Physiol.*, 1954, **62**, 134  
 133 MITCHELL, J. S., *Experientia*, 1949, **5**, 293  
 134 MITCHELL, J. S., *Annu. Rep. Brit. Emp. Cancer Campgn*, 1950, **27**, 214; 1951, **28**, 213  
 135 MITCHELL, J. S., *Brit. J. Cancer*, 1953, **7**, 213  
 136 VERLY, W., KOCH, R. and GRÉGOIRE, S., *Symp. Radiobiol.*, Liège, Butterworths, London, 1955  
 137 LATARJET, R. and GRAY, L. H., *Acta Radiol.*, 1954, **41**, 61  
 138 LANGENDORFF, H., KOCH, R. and SAUER, H., *Strahlentherapie*, 1954, **93**, 281  
 139 HOLLAENDER, A. and DOUDNEY, C. O., *Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955  
 140 LACASSAGNE, A., DUPLAN, J. F. and BUU-HOI, N. P., *Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955  
 141 EICHEL, H. U. and ROTH, J. S., *Biol. Bull.*, 1953, **104**, 351

#### REFERENCES

- <sup>142</sup> BETZ, H., *Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955
- <sup>143</sup> BACQ, Z. M., BEAUMARIAGE, M. L. and FISCHER, P., *Proc. physiol. Soc.*, Oxford, Sept. 1954; *Bull. Acad. Méd. Belg.*, 1954, in press
- <sup>144</sup> LACASSAGNE, A., *Fortschr. Geb. Rontgenstr.*, 1951, **75**, 98
- <sup>145</sup> PATT, H. M., STRAUBE, R. L., TYREE, E. B., SWIFT, M. N. and SMITH, D. E., *Amer. J. Physiol.*, 1949, **159**, 269
- <sup>146</sup> LANGENDORFF, H., KOCH, R. and SAUER, H., *Strahlentherapie*, 1954, **93**, 381
- <sup>147</sup> LANGENDORFF, H. and KOCH, R., *ibid.*, 1954, **94**, 250
- <sup>148</sup> LANGENDORFF, H. and KOCH, R., *ibid.*, 1954, **94**, 411
- <sup>149</sup> KING, E. D., SCHNEIDERMAN, H. A. and SAX, K., *Proc. nat. Acad. Sci.*, 1952, **38**, 34
- <sup>150</sup> GRAY, L. H., *Acta Radiol.*, 1954, **41**, 63
- <sup>151</sup> HALL, B. V., *Argonne nat. Lab.*, ANL-4401, 1951, 130
- <sup>152</sup> READ, J., *Annu. Rep. Brit. Emp. Cancer Campgn*, 1954, **31**, 319
- <sup>153</sup> FORSSBERG, A., *Acta Radiol.*, 1954, **41**, 56
- <sup>154</sup> MIKAELSEN, K., *Proc. nat. Acad. Sci.*, 1954, **40**, 171
- <sup>155</sup> PATT, H. M., *Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955
- <sup>156</sup> WOLFF, E., personal communication
- <sup>157</sup> PATT, H. M., MAYER, S. H., STRAUBE, R. L. and JACKSON, E. M., *J. cell. comp. Physiol.*, 1953, **42**, 327
- <sup>158</sup> MAISIN, J., MAISIN, H. and DUNJIC, A., *Symp. Radiobiol.*, Liège, 1954, Butterworth, London, 1955
- <sup>159</sup> BACQ, Z. M., *Acta Radiol.*, 1954, **41**, 47
- <sup>160</sup> GERSCHMANN, R., NYE, S. W., GILBERT, D. L., DWYER, P. and FENN, W. O., *Proc. Soc. exp. Biol. Med.*, 1954, **85**, 75
- <sup>161</sup> GERSCHMANN, R. and FENN, W. O., *Amer. J. Physiol.*, 1954, **176**, 6
- <sup>162</sup> ELDJARN, L., *Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955
- <sup>163</sup> DALE, W. M., *Biochem. J.*, 1942, **36**, 80
- <sup>164</sup> DALE, W. M., *Trans Faraday Soc., Disc.*, 1952, **12**, 293
- <sup>165</sup> DOHERTY, G. D., *Fed. Proc.*, 1952, **11**(i), 35
- <sup>166</sup> ALEXANDER, P., *Brit. J. Radiol.*, 1953, **26**, 413
- <sup>167</sup> ALEXANDER, P. and FOX, M., *Trans. Faraday Soc.*, 1954, **50**, 605
- <sup>168</sup> ALEXANDER, P. and FOX, M., *J. Chim. phys.*, 1953, **50**, 415

## EFFECTS OF PROTECTION OF PART OF THE MAMMALIAN BODY BY A LEAD SCREEN

**P**HYSICAL protection of part of the body is very useful as a method of studying: (i) The relative importance of the protected organs in the survival of the animal; (ii) the presence or absence of repercussions of irradiation on the protected region; (iii) the influence of the protected parts on the irradiated parts.

It is no longer possible to question that there are reciprocal influences between the irradiated and the protected parts of the body, effected through the neuro-endocrine system, and also through the production of substances in the irradiated tissues and their diffusion into the blood and neighbouring tissues. Publications on this subject are very numerous, and examples have been selected from the recent literature, paying special attention to protection of the spleen, the region of the liver, and a certain proportion of the bone-marrow.

### IRRADIATION OF THE HIND LEGS OF THE RABBIT

A group of investigators at Liège<sup>1-4</sup> confined radiation to the hind legs of the rabbit because these consist of a considerable volume containing neither nerve cells nor endocrine glands, nor any part of the gastro-intestinal tract\*. After a dose of 2000 r to the hind legs a number of effects at a distance are observed. (i) Transitory changes in the oligodendroglia, lymphopenia reaching its maximum in 6 hours, thrombopenia reaching its maximum in no more than 3 hours, slight hydraemia, histological changes in the spleen, cervical lymph glands and damage to non-irradiated bone-marrow. (ii) The number of abnormal megakaryocytes rises from 10 to 50 per cent in 24 hours in both the irradiated and non-irradiated bone-marrow, but whereas the percentage of abnormal cells in the protected tissue falls on the second and third days, it continues to rise in the irradiated tissue. (iii) The increased destruction of lymphocytes is not accompanied by an increase in the circulating antibodies. (iv) An

\* The rest of the body is well protected; this is confirmed by the loss of hair which is always confined to the hind legs.

#### IRRADIATION OF THE HIND LEGS OF THE RABBIT

early discharge of lipids from the adrenal cortex (in 3 hours) is observed, but it is transitory and not followed by hypersecretion of 17-ketosteroids. There are therefore signs of a slight hypophyseoadrenal reaction to stress (see also Chapter 12, p. 272), but it is impossible to say how much of the effect can be attributed to this neuro-endocrine reaction and how much to substances liberated in the irradiated parts. It can be affirmed that these substances have not been invented for the sake of a theory. A neat experiment by JOLLES<sup>5, 6</sup> is not subject to the doubts in interpretation we have mentioned.

In man, squares of skin, 1.5 to 3.5 cm square, are irradiated simultaneously through lead grids. The squares are arranged in a

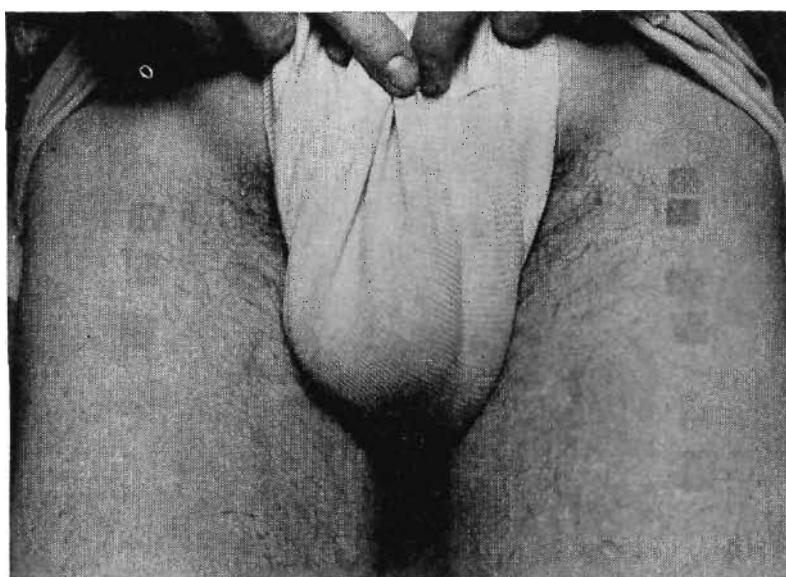


Figure 1. Erythema of human skin, 40 days after irradiation with 1000 r to each pair of squares

single row, various distances (0.25 to 3 cm) apart (Figure 1). If the squares are near enough to each other (2 cm or less for squares of  $2.5 \text{ cm}^2$ ), the intensity of the erythema is distinctly more marked\*. The idea of a diffusible substance in the skin itself seems unavoidable, but if it diffuses in the skin, it is difficult to see why it should not also pass into the blood.

\* This technique of irradiation through a grid is the foundation of research on the importance of connective tissue in the reaction of tumours to irradiation<sup>6</sup>; the fractioning of doses in space is mainly of interest to radiotherapists, and lies outside the scope of this work.

## EFFECTS OF PROTECTION OF MAMMALIAN BODY BY LEAD SCREEN

### PARTIAL IRRADIATION AND THE PRODUCTION OF LYMPHOMATA

A fine series of investigations by KAPLAN<sup>7</sup> shows the influence of normal tissue on the irradiated parts. A certain type of lymphoma appears spontaneously in certain strains of mice, and can also be produced by x-rays, oestrogens and carcinogenic hydrocarbons. The tumour seems to spread from the thymus<sup>8</sup> but local irradiation of the thymus and the regions adjoining it does not cause the appearance of such tumours. The whole mouse must be irradiated, or partial irradiations must follow in rapid succession and cover the whole body. It suffices to keep one thigh protected throughout to cause a striking diminution in the incidence of lymphomata<sup>9, 10</sup> (see *Figure 1*, p. 339).

If one thigh has not been irradiated the behaviour of the thymus is altered. It is possible that this inhibition of the development of lymphoma is brought about by the bone-marrow in this limb<sup>11</sup>, and that Kaplan's findings are therefore to be added to those of Jacobson, Lorenz, and all others who have demonstrated the importance of a small amount of intact haemetopoietic tissue in the recovery or restoration of irradiated mammals.

### PROTECTION OF THE SPLEEN, LIVER, BONES AND OTHER ORGANS

In 1949 Jacobson and his colleagues found that in anaesthetized CF-1 mice, protection of the exteriorized spleen (0·1 g) by a lead screen caused a striking proportion (96 per cent) of survivals after a dose (700 r) of x-rays which killed all controls (anaesthetized mice with the spleen exteriorized but not protected<sup>12, 13</sup>). A dose of 1050 r must be given to animals with a protected spleen to produce about the same death rate as 600 r in controls. Jacobson's technique is logical and exact, and the controls are true controls<sup>13</sup>. However, many authors have failed to confirm Jacobson's original experiment, and others have obtained a less striking and especially a very variable rate of survival<sup>19</sup>. These contradictions are apparently being explained. Jacobson happened, by chance, to carry out his experiments with a particularly favourable strain of mice. When KAPLAN and PAULL<sup>15</sup> repeated Jacobson's experiments they obtained the results shown in *Table I* (see also<sup>45</sup>).

In black C57 mice the difference is hardly significant, whereas in animals of strain A it is of the same order as that found by Jacobson. In rats protection of the spleen by a lead screen does not seem to en-

## PROTECTION OF THE SPLEEN, LIVER, BONES AND OTHER ORGANS

sure a large proportion of survivors<sup>16</sup>, whereas in dogs the experiment is about as successful as in Jacobson's mice<sup>17, 18</sup>. KAPLAN<sup>15</sup> suggests that the differences between different strains may be explained by genetic differences in the haematopoietic activity of the spleen but in dogs the spleen is a purely muscular and lymphoid organ.

*Table I. Influence of Spleen Shielding on Mortality of Mice after 500 r Whole-body Radiation<sup>15</sup>*

	Strain A		Black strain C57	
	No. of animals	Per cent mortality	No. of animals	Per cent mortality
Spleen protected . . .	42	0	35	23
Spleen exteriorized but not protected . . .	43	74	41	54
Intact mice . . .	45	76	44	59

Jacobson's original observation has stimulated a large number of investigations, of which the following are the most interesting:

(i) If spleens which have been protected by a lead screen are excised five minutes after irradiation there are no survivors, but if splenectomy is performed 1 to 6 hours after irradiation the percentage of survivors is considerable (*Table II*). The non-irradiated spleen therefore does not act during irradiation, but during the hours which follow it.

*Table II. Survival of Mice exposed to 1025 r with Lead Protection of Exteriorized Spleen and Splenectomy at various Intervals after Irradiation. After JACOBSON et al.<sup>14</sup>*

No. of Mice	Interval between irradiation ad splenectomy	Survival per cent
24	10 min before irradiation	0
24	5 min after irradiation	0
54	1 to 6 hours after irradiation	66
95	2 days after irradiation	39

(ii) Grafts of homologous spleen *after* irradiation do not always give a satisfactory rate of survival unless everything is arranged in their favour, for example, by transplanting the spleen of from four to nine daughter mice into the abdomen\* of a young mother soon after irradiation<sup>14, 20, 26-28, 40, 45</sup>.

\* Subcutaneous grafts are ineffective<sup>2, 6</sup>. Apparently the splenic factor must pass through the liver in order to be effective. This fact is curiously reminiscent of the late H. Rein's experiments showing the existence in vertebrates of a splenic hormone (anoxyhaemine) which is active only after passing through the liver, and which is supposed to facilitate the reactions of the body to anoxaemia.

## EFFECTS OF PROTECTION OF MAMMALIAN BODY BY LEAD SCREEN

(iii) The injection of homogenates, but not of extracts of spleen after irradiation ensures the survival of a large number of animals (see Chapter 16).

(iv) The favourable effects of cysteine (see p. 293) injected before irradiation are additional to those of physical protection of the spleen<sup>39</sup>.

(v) Several investigators, JACOBSON's team<sup>14, 28</sup>, GERSHON-COHEN's team<sup>29</sup>, MAISIN's team<sup>16, 21-25</sup>, and KAPLAN's team<sup>30</sup>, have tackled the question whether the action of the spleen is specific, and whether considerable rates of survival might be obtained by protecting other organs with lead. Their results may be summarized as follows (*Table III*):

*Table III Survival of Mice exposed to 1025 r x-radiation with Lead Protection of various Tissues. After JACOBSON et al.<sup>14</sup>*

No. of animals	Tissue protected	Survival (per cent)	Regeneration of haemopoietic organs
135	Exteriorized spleen (0·1 g)	77·7	Complete (+ + + +)
143	None	0	0
15	Exteriorized lobe of liver (0·8 g)	33	Almost complete (+ + +)
15	Exteriorized intestine (2·5 g)	26·6	Almost complete (+ + +)
18	Head (3·0 g)*	27·7	Partial (+)
15	Right hind leg with thigh* (1·5 g)	13	Not studied
23	Exteriorized right kidney	0	0

\* See also LAMERTON's results<sup>46</sup>.

(a) Protection of a thigh by a lead screen is effective<sup>46</sup>. Here it is the bone (probably the bone-marrow) which is of consequence, because if the thigh with the bone removed is protected while rats are irradiated, the death rate is the same as in controls<sup>21, 22, 30</sup>. This is important, because in many experiments in which a lead screen is applied to the 'hepatic region', the right lung, or the lower abdomen, it is not only the liver, lung or pelvic organs which are protected, but also the haematopoietic tissue of the ribs, vertebrae, and pelvic bones. The only technique which avoids this source of error is Jacobson's in which the organ is *exteriorized* and protected with lead. Unfortunately this technique requires deep anaesthesia, and makes it important to run careful control experiments †.

† To be accurate it is also necessary to take into account the weight or size of the region protected by the metal screen; for an animal in which part of the body is protected receives a smaller dose than the controls, and if tests are being made near LD<sub>50</sub>, i.e. in a zone of great variability, the fact of giving, say, 50 r less may reduce the death rate considerably<sup>29, 30, 34, 35</sup>.

#### PROTECTION OF THE SPLEEN, LIVER, BONES AND OTHER ORGANS

The rat tail does not contain any haematopoietic bone-marrow, and protecting it with a lead screen does not produce a greater number of survivals than in controls. If the tail is transplanted into the abdomen, the bones in it gradually fill with haematopoietic tissue. If it is then exteriorized and protected with lead, the survival rate after irradiation is better than in controls<sup>42</sup>.

Bone-marrow grafts generally give no results<sup>31, 32</sup>, the effect of homogenates of bone-marrow is discussed in Chapter 16.

(b) Irradiation in man produces radiation sickness very quickly when applied to the upper abdomen (see especially reference<sup>33</sup>), and protection of this region with a lead screen ensures a good proportion of survivals<sup>30, 34, 35</sup>. The liver has attracted the attention of many authors, especially MAISIN<sup>16, 23-25</sup>. Protection of the region of the liver, or still better, of the middle lobe of the liver, especially in animals which have fasted for 24 hours, is claimed to give a very high proportion of survivals<sup>24</sup>. Some authors have found that shielding of the liver has but little effect, although LANGENDORFF *et al.*<sup>45</sup> found that implantation of heterologous liver produced some recovery.

In JACOBSON's experiments<sup>14</sup> (*Table III*), the shielding of an exteriorized lobe of the liver (0.8 g of tissue) gives a greater survival rate than protection of the head (3 g) or of 2.5 g of exteriorized gut, the liver being second only to the spleen among the organs important for the restoration of irradiated mice<sup>14</sup>. STRAUBE and PATT<sup>36</sup> confirm the importance of the liver, but, unlike Maisin, they do not observe any results from injections of cysteamine *after* irradiation.

The hepatic region must not be taken to mean the liver alone (except in<sup>14</sup>). It is possible to exclude the spleen and adrenal, and, if necessary, the ribs and vertebrae, but a fragment of duodenum and pancreas must be included. A general idea seems to be slowly emerging from these investigations, that the protection given to animals by an abdominal screen can be attributed to the summation or synergism of protecting several organs rather than a single organ, such as the spleen, liver or gut<sup>37</sup>.

If a normal rat is connected in parabiosis with a rat which has received a lethal dose of x-rays, the irradiated rat survives<sup>38</sup>. The complete, intact, grafted animal transmits substances or cells in its blood to the irradiated animal, which enables it to overcome the effects of the ionizing radiations.

## EFFECTS OF PROTECTION OF MAMMALIAN BODY BY LEAD SCREEN

### CAUSES FOR THE SURVIVAL OF ANIMALS PARTLY PROTECTED BY SCREENING WITH LEAD

Though opinions differ greatly as to the relative importance of the spleen, liver and bone-marrow, there is complete agreement as to the behaviour of animals which survive as a result of physical protection of part of the body. Whatever school is concerned, JACOBSON's<sup>14</sup> (*Table III*), CRONKITE's<sup>38</sup>, MAISIN's<sup>25, 48</sup>, or any other, all observations record a single fact, namely that *regeneration of the haematopoietic organs is more rapid* in mice or rats in which the thigh, spleen, or liver has been protected. This is also the important phenomenon observed in irradiated animals protected chemically by cyanide, cysteine, or cysteamine (see p. 316). CRONKITE<sup>41</sup> rightly emphasizes this fundamental fact, which draws the attention of radiobiologists to the existence of a regenerative factor in the haematopoietic organs. This point is again considered at the end of Chapter 16.

It has been said that the survival of animals in which part of the haematopoietic system has been protected is simply the result of the presence in the blood stream of a minimum number of normal leucocytes to combat infection. This idea is difficult to reconcile with the investigations of COLE *et al.*<sup>40</sup> (see p. 338), on the remarkable effect of injections of nuclei of spleen cells and BACQ, HERVE and SCHERBER's<sup>43</sup> observations that mice protected by cysteamine survive in spite of the fact that their blood contains practically no leucocytes for 10 days (see p. 317).

#### REFERENCES

- <sup>1</sup> GEREBTZOFF, M. A. and HERVE, A., *C.R. Soc. Biol.*, 1949, **143**, 880
- <sup>2</sup> BETZ, H. and LECOMTE, J., *ibid.*, 1950, **144**, 303
- <sup>3</sup> LECOMTE, J. and FISCHER, P., *ibid.*, 1949, **143**, 878
- <sup>4</sup> NIZET, E., HEUSGHEM, C. and HERVE, A., *ibid.*, 1949, **143**, 876
- <sup>5</sup> JOLLES, B., *Brit. J. Radiol.*, 1950, **23**, 18
- <sup>6</sup> JOLLES, B., *X-ray Sieve Therapy in Cancer*, Lewis, London, 1953, p. 192
- <sup>7</sup> KAPLAN, H. S., *Acta Un. int. Cancer*, 1952, **7**, 849
- <sup>8</sup> KAPLAN, H. S., *J. nat. Cancer Inst.*, 1948, **8**, 191
- <sup>9</sup> KAPLAN, H. S. and BROWN, M. B., *ibid.*, 1951, **12**, 427
- <sup>10</sup> KAPLAN, H. S. and BROWN, M. B., *Cancer Res.*, 1952, **12**, 441
- <sup>11</sup> KAPLAN, H. S. and BROWN, M. B., *Science*, 1952, **116**, 195
- <sup>12</sup> JACOBSON, L. O., MARKS, E. K., GASTON, E. O., ROBSON, M. and ZIRKLE, R. E., *Proc. Soc. exp. Biol. Med.*, 1949, **70**, 740
- <sup>13</sup> JACOBSON, L. O., MARKS, E. K., ROBSON, M. J., GASTON, E. and ZIRKLE, R. E., *J. Lab. clin. Med.*, 1949, **34**, 1538
- <sup>14</sup> JACOBSON, L. O., SIMMONS, E. L., MARKS, E. K. and ELDREDGE, J. H., *Science*, 1951, **113**, 510

#### REFERENCES

- <sup>15</sup> KAPLAN, H. S. and PAULL, J., *Proc. Soc. exp. Biol. Med.*, 1952, **79**, 670
- <sup>16</sup> MANDART, M., LAMBERT, G., MAISIN, H. and MAISIN, J., *C. R. Soc. Biol.*, 1952, **146**, 1647
- <sup>17</sup> COULTER, M. P. and FURTH, F. W., *Univ. Rochester Atom. Energy Proj.*, UR-189, 25 Oct. 1951
- <sup>18</sup> INGRAM, M., NIELSEN, G. and PIATT, D., *ibid.*, UR-200, 11 Feb. 1952
- <sup>19</sup> BRUES, A. M., *Argonne nat. Lab.*, ANL-4571, 1951
- <sup>20</sup> MANDART, M., LAMBERT, G. and MAISIN, J., *C.R. Soc. Biol.*, 1952, **146**, 1305, 1307
- <sup>21</sup> MANDART, M., LAMBERT, G. and MAISIN, J., *ibid.*, 1952, **146**, 1392
- <sup>22</sup> MANDART, M., LAMBERT, G. and MAISIN, J., *ibid.*, 1952, **146**, 1645
- <sup>23</sup> MAISIN, J., MANDART, M., LAMBERT, G. and MAISIN, H., *ibid.*, 1953, **147**, 362
- <sup>24</sup> MAISIN, J., DUNJIC, A., VAN LANCKER, J., LAMBERT, G. and PASSEAU, J., *ibid.*, 1953, **147**, 1517, 1520
- <sup>25</sup> VAN LANCKER, J., *ibid.*, 1953, **147**, 1513, 1515, 1523
- <sup>26</sup> PETZ, H., personal communication
- <sup>27</sup> BARNES, D. W. H. and LOUITT, J. F., *Proc. roy. Soc. Med.*, 1953, **46**, 251
- <sup>28</sup> JACOBSON, L. O., MARKS, E. K., ROBSON, M. J., GASTON, E. and ZIRKLE, R. E., *J. Lab. clin. Med.*, 1951, **37**, 683
- <sup>29</sup> GERSHON-COHEN, J., HERMEL, M. B. and GRIFFITH, J. Q. Jr., *Science*, 1951, **114**, 157
- <sup>30</sup> KAPLAN, H. S., see *Nucleonics*, 1951, **8**, No. 4, 33
- <sup>31</sup> BRUES, A. M., *Argonne nat. Lab.*, ANL-4625, April 1951
- <sup>32</sup> STORER, J. B., AECU-2095, 1952
- <sup>33</sup> HERVE, A., *J. belge Radiol.*, 1952, **35**, 655
- <sup>34</sup> COULTER, M. and MILLER, M., *Univ. Rochester Atom. Energy Proj.*, UR-205, 14 May 1952
- <sup>35</sup> BLAIR, H. A., *ibid.*, UR-205, 31 August 1952
- <sup>36</sup> STRAUBE, R. L. and PATT, H. M., *Proc. Soc. exp. Biol. Med.*, 1953, **84**, 702
- <sup>37</sup> FARR, R. S. and BRUYN, P. P. H., AECU-1820, Oct. 1951
- <sup>38</sup> BRECHER, G. and CRONKITE, E. P., *Proc. Soc. exp. Biol. Med.*, 1951, **77**, 292
- <sup>39</sup> BRUES, A. M., *Argonne nat. Lab.*, ANL-4571, 1951
- <sup>40</sup> COLE, L. J., FISHLER, M. C., ELLIS, M. E. and BOND, V. P., *Proc. Soc. exp. Biol. Med.*, 1952, **80**, 112
- <sup>41</sup> CRONKITE, E. P., *Atomic Medicine*, Williams and Wilkins, Baltimore, 1953, 2nd edn, p. 178
- <sup>42</sup> STORER, J. B., LUSHBAUGH, C. C. and FURCHNER, J. E., *J. Lab. clin. Med.*, 1952, **40**, 355
- <sup>43</sup> BACQ, Z. M., HERVE, A. and SCHERBER, F., *Arch. int. Pharm. Thér.*, 1953, **94**, 93
- <sup>44</sup> *J. Amer. med. Ass.*, Editorial, 27 Dec. 1952, p. 1673
- <sup>45</sup> LANGENDORFF, H., KOCH, R. and SAUER, H., *Strahlentherapie*, 1954, **93**, 274
- <sup>46</sup> LAMERTON, L. F., *Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955
- <sup>47</sup> JACOBSON, L. O., *ibid.*
- <sup>48</sup> MAISIN, J., MAISIN, H. and DUNJIC, A., *ibid.*

## INJECTIONS OF HOMOGENATES OF SPLEEN OR BONE-MARROW AFTER IRRADIATION\*

METHODS of investigation using partial protection with lead screens are informative, but they cannot answer the fundamental question as to what is responsible for the activity of the spleen, bone-marrow or liver, and whether it is a substance produced by these tissues or cell migration, a swarming starting from non-irradiated organs.

Much valuable information has been obtained from experiments with injection of extracts, or of complete or fractioned homogenates from different organs†, and the pertinent experiments are summarized below.

(a) Injections of extracts made by the standard, destructive methods produce no results at all<sup>1</sup>.

(b) Injections of homogenates‡ or suspensions of bone-marrow taken from an animal of the same, or even of a different species result in the survival of a large proportion of animals (mice, guinea-pigs) which have received a lethal dose of x-rays. The injections are given intraperitoneally or intravenously *after* irradiation. This fact has been confirmed several times during the last three years by different groups of authors (LORENZ *et al.*<sup>2-4</sup>, BRUES<sup>5</sup>, BROWN, KAPLAN *et al.*<sup>6</sup> §).

\* It is unfortunate that several authors<sup>1, 7-9</sup> speak of *protection* of rodents against x-rays when they mean action after irradiation, often several days after it, and when radiation sickness is fully established. A better term is *treatment*, or modification of the effects of x-rays<sup>3, 4, 6, 11</sup> for action taken *after* irradiation, and one must retain the term *protection* for physical, chemical or biological measures taken *before* and during irradiation. We agree with the definitions for these terms which have been proposed by Latarjet and Gray<sup>18</sup>.

† For technical reason experiments so far have been carried out only in rats, mice and guinea pigs.

‡ Readers interested in the history of medicine will find a full account of the literature on injections and grafts of bone-marrow in man and animals in CONGDON, UPHOFF and LORENZ<sup>4</sup>.

§ The value of homogenates is linked with the development of modern cytochemistry, in which the pioneers were Claude, Potter, Elvehjem, Brachet, Hogeboom, Schneider, and others. The cell membranes are destroyed, but the intracellular structures (nucleus, mitochondria, microsomes) are left intact. Homogenization can be achieved more or less perfectly by various types of apparatus. Mechanical mixers or blenders, such as are also used in the kitchen, do not preserve the nuclei. Grinding in a mortar with quartz sand is a blind method, leaving some cells unbroken and damaging the intracellular structures.

#### INJECTIONS OF HOMOGENATES OF SPLEEN OR BONE-MARROW

(c) Homogenates of mouse spleen are active if injected 1 to 45 hours after irradiation<sup>8, 15</sup>. Generally a single intraperitoneal injection is given, 2 to 3 hours after irradiation.

As much restoration is produced in mice which have received a lethal dose of irradiation by a homogenate of 28 mg of spleen from 8-day old mice as by a homogenate of 100 mg of spleen from adult mice<sup>15</sup>. If one injects  $3 \times 10^6$  cells (suspended in saline) from haematopoietic organs of young donors the survival is 63 per cent in mice irradiated with 900 r as compared with 23 per cent when the irradiated mice were injected with the same number of bone-marrow cells from adult donors<sup>20</sup>. With 750 r, only 150,000 marrow cells from 4- to 5-week old mice are required to enhance survival<sup>20</sup>.

The effect of homogenates after irradiation is more marked in adult than in very young mice. These homogenates are also effective in splenectomized mice which have been irradiated<sup>15</sup>. Genetic factors play some part; for example, homogenates of spleen from mice of the LAF<sub>2</sub> strain are not effective for irradiated LAF<sub>1</sub> mice<sup>15</sup>.

If homogenates of spleen from young mice are subjected to fractional ultracentrifugation in isotonic sucrose with phosphates and ATP, it is found that the active substance is confined entirely to nuclei. The presence of adenosine triphosphoric acid (ATP) and phosphates in the isotonic sucrose solution adds considerably to the activity of the isolated nuclei. This suggests that ATP is needed either for the maintenance of the semipermeability of the nuclear membrane, or for the integrity of the intranuclear growth factor. The mitochondria, microsomes and supernatant fluid are quite ineffective<sup>9</sup> (see *Table I*). If the structure of the nucleus is destroyed by ultrasonic vibrations, or if the nuclei are irradiated, the effect

---

The Potter-Elvehjem grinder is the most satisfactory, both in principle and in practice, the tissue being forced between a piston and cylinder wall, accurately calibrated so that the distance between them is less than the smallest diameter of the cells, but greater than that of the nucleus.

Both homogenization and the manipulations needed to separate the various fractions by ultracentrifugation at increasing speeds must be carried out at a temperature near 0° C. The ground tissue is diluted with a hypertonic solution of sucrose (with any desired modifications), which prevents rupture of the intracellular structures. The first centrifugation separates the nuclei and remnants of cell membranes, the second separates the mitochondria, the third the large microsomes, and so on until there are no more particles denser than the sucrose solution. What is left after repeated ultracentrifugation is called the supernatant fluid.

The techniques of homogenization and fractional centrifugation are used in research on the intracellular distribution of enzymes, and also of fragile substances, such as the nucleo-proteins, which are destroyed during extraction by the standard, destructive methods.

### INJECTIONS OF HOMOGENATES OF SPLEEN OR BONE-MARROW

disappears. This suggests that the active substance, the unidentified factor for regeneration of the haematopoietic tissues, is a highly polymerized nucleoprotein, rather fragile, and certainly radiosensitive<sup>9</sup>. Nucleic acid extracted from spleen nuclei by 1M NaCl, which splits the nucleoprotein (see p. 145), is quite inactive.

*Table I. Percentage of Survivals 30 days after Irradiation (750 r) in Mice after Intraperitoneal Injection of Homogenates or Fractions of Homogenates of Spleen 2 to 3 hours after Irradiation. After COLE, FICHLER and BOND<sup>9</sup>*

<i>Fraction injected</i>	<i>Equivalent quantity of spleen tissue mg</i>	<i>Survival per cent</i>
<i>Total homogenate in sucrose-saline medium*</i>	56	41 (7/17)
<i>Nuclei from this homogenate</i>	280	100 (9/9)
<i>Mitochondria</i>	250	0 (0/8)
<i>Microsomes + supernatant fluid</i>	140	0 (0/8)
<i>Sucrose + salt soln</i>	—	0 (0/10)
 <i>Homogenate in sucrose-saline medium</i>	 56	 100 (10/10)
<i>Nuclei from this homogenate</i>	280	100 (10/10)
<i>Mitochondria*</i>	250	0 (0/8)
 <i>Homogenate in sucrose-saline medium</i>	 39	 100 (5/5)
<i>Nuclei</i>	168	38 (3/8)
<i>Nuclei in 1M NaCl</i>	150	0 (0/6)
<i>Controls</i>	—	0 (0/11)
 <i>Homogenate in sucrose-saline medium</i>	 56	 87 (7/8)
<i>Nuclei</i>	196	50 (2/4)
<i>Sucrose-saline soln</i>	—	0 (0/7)
 <i>Homogenate of spleen</i>	 56	 70 (7/10)
<i>Same exposed to 750 r</i>	56	0 (0/10)
<i>Phosphate buffer</i>	—	0 (0/10)

\* 0.242M sucrose, 0.0094M KH<sub>2</sub>PO<sub>4</sub>, 0.0125M K<sub>2</sub>HPO<sub>4</sub>; 0.0015M NaHCO<sub>3</sub> and 0.0006M NaATP.

† Intravenous injection.

(Injections of homogenates of spleen or bone-marrow after irradiation.)

Recently COLE and ELLIS<sup>10</sup> showed that the spleen homogenate or nuclei is inactivated (*i.e.* no longer increases the survival time of irradiated mice) by incubation with a proteolytic enzyme, trypsin, or by treatment with deoxyribonuclease which attacks DNA. Ribonuclease which attacks only RNA brought about no inactivation. As living cells are not acted upon by enzymes this work indicates that the active principle is non-cellular and probably a very fragile DNA-protein complex.

If these results are compared with those given by protection of the spleen and bones by a lead screen, the conclusion is reached that the haematopoietic organs promote the regeneration of

## INJECTIONS OF HOMOGENATES OF SPLEEN OR BONE-MARROW

irradiated tissues not by sending out cells, but by allowing the gradual diffusion from their nuclei of a fragile substance, which can only persist in the intact nuclei.

(d) Intravenous injections of suspensions of bone marrow cells inhibit the development of lymphoma in mice after total irradiation<sup>11</sup>, and promote regeneration of the thymus<sup>6</sup> (*Figure 1*).

(e) All the evidence suggests that the active factor in spleen homogenates is the same as that in bone-marrow<sup>7, 9</sup>. In mice, and to a lesser extent in rats, the spleen must be regarded as a complete haematopoietic organ, which produces not only lymphocytes and erythrocytes, but also granulocytes and megakaryocytes\*<sup>7</sup>. For

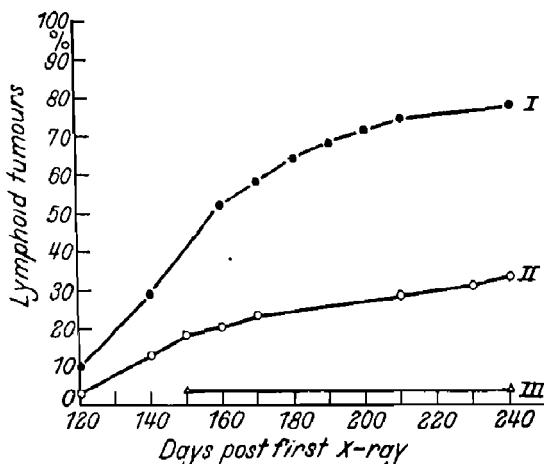


Figure 1. Effect of intravenous bone-marrow cell suspensions on development of radiation-induced lymphoma in strain C-57 black mice<sup>11</sup>. I. x-irradiation only; II. x-irradiation + bone-marrow injection; III x-irradiation with thigh shielded

instance, in KAPLAN's experiments<sup>11</sup> the active principle in the suspensions of bone-marrow is not easily soluble in water, or else it is destroyed during extraction. It is radiosensitive, that is, it disappears if the suspension is irradiated before injection. It must be injected fairly soon after irradiation. The best results are obtained if it is injected on about the third or fourth day. A bone-marrow injection given on the twelfth day is ineffective. Injections of bone-marrow into the peritoneum are less effective than intravenous injections.

\* Homogenates of the bone-marrow of rats are more active than those of the spleen of the same species, probably because the spleen is not such an important haematopoietic organ in rats as in mice.

#### INJECTIONS OF HOMOGENATES OF SPLEEN OR BONE-MARROW

(f) A considerable amount of homogenized bone-marrow or spleen must be injected in order to obtain a significant rate of survival<sup>8</sup>. For instance, in order to reduce the death rate in rats after irradiation with 700 to 725 r from 100 to 30 per cent, a suspension of 50 to 100 mg of bone-marrow must be injected intravenously. To obtain the same result after a dose of 750 to 770 r the quantity of bone-marrow must be increased to 200 mg. After 800 r, even 400 mg of bone-marrow injected after irradiation are ineffective, probably because rats which have received 800 r die from diarrhoea and from an effect on the intestine against which the injection of bone marrow has no effect <sup>7, 12</sup>.

(g) The active substance is not specific for a given species. For instance, injections of a suspension of guinea-pig bone-marrow result in the survival of 40 to 60 per cent of mice irradiated with a dose (800 r) of x-rays which kills all the controls<sup>4</sup>. In this experiment it is difficult to sustain the alternative view that the animals survive because *intact cells* become implanted in the body into which they are injected.

(h) Intravenous injections of spleen homogenates into mice irradiated with sub-lethal doses increase their resistance to *B. proteus*; the effect is additive to that brought about by streptomycin<sup>13</sup>.

(i) Finally, the nuclei obtained from mouse spleen are very diverse; in addition to the mother cells of the circulating blood cells, cells of the reticulo-endothelial system are present in the spleen and bone-marrow. CONGDON, UPHOFF and LORENZ<sup>4</sup> say, in support of the idea that the reticulo-endothelial cells are important, that in a few preliminary experiments injections of a suspension of cells from a well-differentiated reticulo-sarcoma prolonged the life of irradiated mice. As far as we know, no systematic search for a regenerative factor in liver homogenates has yet been made, but it seems that foetal (haematopoietic) liver is active, unlike adult liver, which is still rich in reticulo-endothelial cells<sup>14</sup>. Homogenates or suspensions of thymus and striated muscle are inactive.

The effect of these injections of bone-marrow or spleen seems to be the same as that of physical protection of the spleen, or of a femur of the thoracic cage, by a lead screen: that is, they greatly accelerate the regeneration of the haematopoietic system<sup>4</sup>. This parallelism may possibly be extended even further.

#### WORKING HYPOTHESES

The experiments of Gerebtzoff and Bacq (see p. 320) show clearly that chemical protection with mercaptoethylamine has little influence

#### WORKING HYPOTHESES

on reactions of the thymus, accelerates regeneration of the intestine and distinctly diminishes the primary changes in the spleen and liver. Cronkite as well as the present authors have assumed that the essential effect of chemical protectors is to prevent the destruction of a factor which promotes regeneration of the haematopoietic system, and whose action does not become evident until 3 to 4 days after irradiation. There is no reason why we should not suppose, as a working hypothesis, that this hypothetical factor is the same as the factor in the nuclei of the spleen and bone-marrow cells, whose action does not become evident for at least as long.

A relationship must also be sought between the results of physical protection and injections of homogenates of spleen and bone-marrow on the one hand, and the experiments which demonstrate intense anabolic and catabolic activity in the bone-marrow and reticulocytes immediately after irradiation on the other (see p. 248).

The following hypothesis emerges: several steps have already been demonstrated although a number of observations remain to be fitted into place:

(i) Irradiation increases the permeability of the cellular membranes (see p. 185).

(ii) The factor for growth regeneration (nucleoprotein *X* of Cole *et al.*) contained in the nuclei of haematopoietic organs *normally* filters out of the nucleus only in very small quantities, but might, during and immediately after irradiation, leave the nucleus *en masse*.

(iii) This factor *X* would thus come into contact with the enzymes and substrates of the cytoplasm, which would cause an enormous increase in biochemical activity at this point (see p. 245).

(iv) The formation, or resynthesis of this factor must be assumed to be slow in normal conditions and in any case practically impossible after irradiation, whence the early arrest of haematopoietic activity which is well known to cytologists and biochemists.

(v) Factor *X*, once it has emerged from the nucleus, is very fragile and is very quickly destroyed. It passes into the blood, where its effect on the reticulocytes can be demonstrated (Nizet, Lambert, Herve and Bacq, see p. 250).

(vi) The only way to help the haematopoietic system of an irradiated animal is to give it a sufficient quantity of factor *X* in the form of homogenates of nuclei of spleen or bone-marrow.

This hypothesis is not incompatible with the fact that radiosensitivity is *increased* in polycythaemic rats during the reticulocytic reaction to a prolonged stay at an atmospheric pressure reduced to 380 mm Hg<sup>16</sup>. For example, the mortality after 760 r, which is 48 per cent in normal rats, rises to 78 per cent in polycythaemic

#### INJECTIONS OF HOMOGENATES OF SPLEEN OR BONE-MARROW

rats irradiated at normal atmospheric pressure (760 mm Hg) and kept at this pressure after irradiation, and to 87 per cent in polycythaemic rats irradiated at normal atmospheric pressure but returned to 380 mm Hg after irradiation. A curious fact is that these polycythaemic rats die with the haemoglobin content of the blood diminished, but equal to or even a little higher than that of controls. The haematopoietic system of irradiated mice no longer responds to chronic anoxia from decompression<sup>17</sup>.

In the present state of our knowledge there is nothing against the supposition that the factor *X* demonstrated by the radiobiologists is also the factor (unknown to the physiopathologists) which stimulates haematopoiesis in all prolonged anoxias. It is possible that chronic anoxia, like ionizing radiations, may promote the diffusion of this factor from the nuclei. An organism in a state of chronic anoxia\* would be more radiosensitive because it would consume more of this substance, and have less in reserve than a normal animal or the synthesis of this substance might be more difficult owing to a decrease in the energy supplied by aerobic glycolysis. BRACHET<sup>19</sup> has shown that the nucleus of *Amoeba proteus* controls the capacity of that organism to keep a high level of ATP in phosphorylated form during anaerobiosis; in the absence of a nucleus, the level of ATP falls in anaerobiosis, probably because glycogenolysis is reduced. Brachet believes that the nucleus plays a part in the activation of glycolytic enzymes probably through the production of DPN (diphosphopyridine nucleotide or coenzyme I).

We have seen on p. 243, that inhibition of oxidative phosphorylations is one of the rare early and consistent biochemical changes observed in spleen homogenates after irradiation, and that glycogen accumulation also occurs in the liver after irradiation. Thus it would be a reasonable working hypothesis to consider a lack of DPN or TPN (triphosphopyridine nucleotide) in the cell as one of the early biochemical lesions following nuclear damage. DPN might escape from the cell and not be resynthetized.

The main experimental objections to this hypothesis are:

(i) Factor *X* is radiosensitive, being destroyed by 750 r *in vitro*, yet we have postulated that it is released into the blood as a result of irradiation. It is, however, possible that this factor is less radiosensitive *in vivo* than *in vitro*, following on the rather drastic extraction from the splenic tissue. It must be admitted that the identification of the factor which increases haemoglobin synthesis by the reticulocytes after irradiation with factor *X* in homogenates of the bone-

---

\* Not to be confused with *acute* anoxia, which, by diminishing the oxygen tension, increases resistance to radiations (see Chapter 8).

### WORKING HYPOTHESES

marrow and spleen of mice is the weakest part of this hypothesis and it is hoped that current work may throw more light on this problem.

(ii) All the experimental evidence for a regeneration factor is confined to the haematopoietic system. This is a special case, but it is of great interest as regards man, because it is possible to transfer results from rodents to all mammals without too much danger of error. Whether this hypothesis can be extended to birds and cold-blooded vertebrates and whether the existence of a regeneration factor is the reason, or one of the reasons, for the extreme radiosensitivity of vertebrates, are problems which can now be approached by systematic experiments.

### REFERENCES

- <sup>1</sup> BARNES, D. W. H and LOUTIT, J. F., *Proc. R. Soc. Med.*, 1953, **46**, 251
- <sup>2</sup> LORENZ, E., UPHOFF, D., REID, T. R. and SHELTON, E., *J. nat. Cancer Inst.*, 1951, **12**, 197
- <sup>3</sup> LORENZ, E., CONGDON, CH. and UPHOFF, D., *Radiology*, 1952, **58**, 863
- <sup>4</sup> CONGDON, C. C., UPHOFF, D. and LORENZ, E., *J. nat. Cancer Inst.*, 1952, **13**, 73
- <sup>5</sup> BRUES, A. M., *Argonne nat. Lab.*, ANL-4625, 1951
- <sup>6</sup> BROWN, M. B., KAPLAN, H. S., WEYMOUTH, P. P. and PAULL, J., *Science*, 1953, **117**, 693
- <sup>7</sup> FISCHLER, M. C., COLE, L. J., BOND, V. P. and MILNE, W. L., *U.S. Naval Radiol. Def. Lab.*, USNRDL-410, 1953
- <sup>8</sup> COLE, L. J., FISCHLER, M. C., ELLIS, M. E. and BOND, N. P., USNRDL-339, 1952; *Proc. Soc. exp. Biol. Med.*, 1952, **80**, 112
- <sup>9</sup> COLE, L. J., FISCHLER, M. C. and BOND, V. P., *Proc. nat. Acad. Sci.*, 1953, **39**, No. 8, 759
- <sup>10</sup> COLE, L. J. and ELLIS, M. E., *U.S. Atom. Energy Rep.*, USNRDL-418, Nov. 23, 1953; *Radiation Res.*, 1954, **1**, 345
- <sup>11</sup> KAPLAN, H. S., BROWN, M. B. and PAULL, J., *J. nat. Cancer Inst.*, 1953, **14**, 303
- <sup>12</sup> BOND, V. P., COLE, L. J., SWIFT, M. N. and FISHLER, M. C., *Aarhus Symp.*, 1953
- <sup>13</sup> MARSTON, R. Q., RUTH, H. J. and SMITH, W. W., *Proc. Soc. exp. Biol. Med.*, 1953, **83**, 289
- <sup>14</sup> LORENZ, E., private communication
- <sup>15</sup> COLE, L. J. and ELLIS, M. E., *Amer. J. Physiol.*, 1953, **173**, 487
- <sup>16</sup> SMITH, W. W., COOL, W. S., SMITH, F. and ALTLAND, P. D., *Amer. J. Physiol.*, 1952, **170**, 396
- <sup>17</sup> SMITH, W. W., DOOLEY, R. and THOMPSON, E. C., *J. Aviat. Med.*, 1948, **19**, 227
- <sup>18</sup> LATARJET, R. and GRAY, L. H., *Acta Radiol.*, 1954, **41**, 61
- <sup>19</sup> BRACHET, J., *Nature, Lond.*, 1954, **173**, 725
- <sup>20</sup> JACOBSON, L. O., MARKS, E. K. and GASTON, E. O., *Symp. Radiobiol.*, Liège, 1954, Butterworths, London, 1955

## OBSERVATIONS IN HUMAN BEINGS

THE study of the general effects of ionizing radiations on man is based on three series of observations: (i) cancer patients treated by local irradiation, (ii) the results of the atomic explosions at Hiroshima and Nagasaki, and (iii) accidents in workers constantly handling radioactive substances or operating generators of ionizing radiations for several years, or employees in atomic energy research establishments who may, in error, receive a heavy dose, which is usually irregularly distributed and which may or may not be lethal. The danger of atomic experimentation, in peace or in war, is not negligible for the individual, and may have even more serious genetic consequences.

### RADIATION SICKNESS

*Radiation sickness in irradiated cancer patients (spondylitis, reticulosis etc.)* has only been studied seriously for a few years, though its existence has been known for fifty years. The facts that irradiation is often carried out in seriously ill patients, and that it is difficult to eliminate psychological influences, both in the patient and in the physician, have placed radiation sickness among that group of symptoms about which practitioners speak without conviction, and which are only rarely discussed seriously. Some say that radiation sickness is very rare, others think that with careful questioning and study of the irradiated patients 20 to 25 per cent will be found to suffer from radiation sickness, of which the symptoms can be summarized as follows:

- Anorexia, nausea, vomiting, diarrhoea;
- Headache, vertigo, insomnia;
- Asthenia, hypotension, cardiac trouble and cardiovascular collapse.

These symptoms are also found after lethal or near lethal irradiation of healthy men (see p. 348). They are now being seriously studied. For equal quantities of energy absorbed, radiation sickness is much more frequent, severe and rapid after irradiation of the abdomen than after irradiation of the chest<sup>1</sup>. ELLIS<sup>2</sup> shows how the value

of treatment in radiation sickness can be evaluated. COURT BROWN and MAHLER<sup>3-5</sup> include these symptoms in the broad syndrome of changes caused by radiations. They pay special attention to the early symptoms, which appear during the first days (or hours) after a therapeutic dose of x-rays. An increase in the quantity of urine with increased loss of NaCl and phosphate is observed as soon as the first symptoms appear. The behaviour of potassium salts is more variable. These observations support the idea that radiation sickness after local irradiation affects the whole body. Mercaptoethylamine (or cysteamine), intravenously, or cystamine by mouth, given after irradiation alleviates the symptoms of radiation sickness<sup>6-8</sup>, *Tables I and II.*

*Table I. Effect of Cysteamine on various Symptoms of Radiation Sickness.  
(BACQ and HERVE<sup>6</sup>)*

	Cases	Cases		Cases	Cases
<i>Anorexia</i>	60	$\begin{cases} 37 = ++ \\ 20 = + \\ 3 = o \end{cases}$	<i>Vertigo</i>	9	$\begin{cases} 8 = + \\ 1 = o \end{cases}$
<i>Nausea</i>	64	$\begin{cases} 55 = ++ \\ 6 = + \\ 2 = o \end{cases}$	<i>Headache</i>	3	$3 = +$
<i>Vomiting</i>	31	$31 = ++$	<i>Colic</i>	1	$1 = ++$
<i>Diarrhoea</i>	19	$\begin{cases} 18 = ++ \\ 1 = + \\ 2 = + \end{cases}$	<i>Insomnia</i>	5	$5 = ++$
<i>Asthenia</i>	16	$\begin{cases} 7 = ++ \\ 7 = o \end{cases}$			

$++$  Symptom disappeared.

$+$  Improvement.

$o$  No change.

*Table II. Response of Radiation Sickness to Cysteamine (BACQ and HERVE<sup>6</sup>)*

<i>Complete digestive syndrome and at least 2 other symptoms</i>	<i>Digestive syndrome with vomiting</i>	<i>Digestive syndrome without vomiting</i>	<i>Monosymptomatic forms (mainly digestive)</i>
17 severe cases	22 moderately severe cases	25 slight cases	15 monosymptomatic cases
2 excellent results	12 excellent results	11 excellent results	11 excellent results
7 good results	7 good results	12 good results	
8 incomplete results	1 incomplete result 2 failures	2 failures	2 incomplete results 2 failures

#### OBSERVATIONS IN HUMAN BEINGS

We emphasize that there is no danger in using cysteamine or cystamine in radiation sickness, *provided they are given after irradiation*. If these protectors are injected immediately before irradiation there is a risk that the destructive effect of the rays on the tumour may be diminished. It has been fully established that these amines are quickly eliminated and metabolized (see p. 299). They disappear in a few hours, and the end products of their oxidation (taurine and sulphate) do not alter the action of x-rays in any way. Neither in animal experiments nor in man is it possible to induce permanent resistance to radiations by means of protectors. The protective effect disappears with the molecule, and this agrees perfectly with the mechanism of action which we have proposed, namely that the protective action of cysteamine is attributable to competition for free radicals (notably the HO<sub>2</sub> radical) formed during irradiation, which have an extremely short life (see p. 314).

In our opinion, the effect of cysteamine and cystamine in radiation sickness when they are administered after irradiation is not related to the purely physicochemical protective action. It is the metabolic actions of these amines, quite apart from any effect of the radiations, which are responsible for their therapeutic effect. Radiation sickness is a generalized syndrome, a sign of lesions at a distance from the irradiated focus. Cysteamine has a favourable effect on these distant reactions of non-irradiated tissues, for example, the vomiting centre. Ignorance of the pathogenesis of radiation sickness prevents our studying the therapeutic effect of cysteamine more closely. Prolonged clinical experience in Belgium bears witness to the usefulness of cysteamine and cystamine, and to the absence of untoward secondary effects<sup>30, 31</sup>.

*A single heavy dose*—Examination of the effects of a single heavy dose such as was experienced in Japan, supposing that there are no burns or other injuries to complicate the clinical picture\*, allows the victims to be very quickly divided into three groups.

(i) Those in whom intractable vomiting sets in a few hours after the explosion. Severe prostration quickly follows; there is complete anorexia and diarrhoea, and a rise in temperature follow. After only 48 hours there is extreme leucopenia, and death occurs within a few days. These have received a whole-body dose of 600 r or more.

(ii) Vomiting occurs *only* on the day of irradiation. After one to three weeks without any clinical symptoms, epilation, purpura, diarrhoea, often with blood in the stools, cutaneous and buccopharyngeal ulceration, infection and anaemia set in. These have

---

\* It is estimated that burns and injuries caused 80 per cent of the deaths in the two atomic explosions of 1945, and radiation sickness only 20 per cent.

### RADIATION SICKNESS

received a dose of 300 to 400 r, and their chances of survival are slender, especially if the infection is not carefully treated.

(iii) There is no vomiting on the day of the explosion. The symptoms are the same as in group (ii), but less severe. There is no leucopenia, or only slight leucopenia about the tenth day. These patients survive even without special care if complicating burns and injuries are not to be feared. They have received about 100 to 200 r.

All attempts at treatment for radiation sickness should in the case of war (or a radiation catastrophe where the number of victims is high) be concentrated on individuals falling into group (ii). *Table III* summarizes the observations made in Japan.

*Table III Summary of Clinical Symptoms of Radiation Sickness<sup>32</sup>*

<i>Time after exposure</i>	<i>Lethal dose (600 r)</i>	<i>Median lethal dose (400 r)</i>	<i>Moderate dose (300–100 r)</i>
	<i>Nausea and vomiting after 1–2 hours</i>	<i>Nausea and vomiting after 1–2 hours</i>	
<i>First week</i>	<i>No definite symptoms</i>		
	<i>Diarrhoea</i> <i>Vomiting</i> <i>Inflammation of mouth and throat</i>	<i>No definite symptoms</i>	
<i>Second week</i>	<i>Fever</i> <i>Rapid emaciation</i> <i>Death</i> <i>(Mortality probably 100 per cent)</i>		<i>No definite symptoms</i>
		<i>Beginning of epilation</i> <i>Loss of appetite and general malaise</i>	
<i>Third week</i>		<i>Fever</i> <i>Severe inflammation of mouth and throat</i>	<i>Epilation</i> <i>Loss of appetite and general malaise</i> <i>Sore throat</i>
<i>Fourth week</i>		<i>Pallor</i> <i>Petechiae, diarrhoea</i> <i>nose bleeds</i>  <i>Rapid emaciation</i> <i>Death</i> <i>(Mortality probably 50 per cent)</i>	<i>Pallor</i> <i>Petechiae</i> <i>Diarrhoea</i> <i>Moderate emaciation</i> <i>(Recovery likely unless complicated by poor previous health or superimposed injuries or infections)</i>

A few cases of *accidental massive irradiation* of nuclear physicists at the Los Alamos research station have been studied and treated with

#### OBSERVATIONS IN HUMAN BEINGS

the greatest care<sup>9</sup>. The two severe cases (in one case probably as much as 600 r whole-body irradiation) ended in death, one on the ninth and one on the twenty-fourth day. They showed the typical symptoms of group (i) : nausea and vomiting within an hour of the accident. The hands, which had received between 5000 and 40,000 r, were affected with sensory disturbances within a few minutes and oedema within an hour \*. Thus the first symptoms of radiation sickness are nervous, although paradoxically the nervous system is usually claimed to be extremely resistant to radiations†. The so-called 'toxic' period set in during the first week in these two cases, with very high temperatures, severe gastro-intestinal disturbances, and severe loss of weight. The patient who died on the ninth day suffered from cardiovascular collapse, jaundice, haemorrhages, and paralytic ileus. The inefficacy of treatment (whole blood, plasma, penicillin, electrolytes etc.) was obvious, and there is little hope of saving severely irradiated patients of this type until better therapeutic measures are discovered. None of the treatments which have been tried with some success in animals (homogenates of bone-marrow or spleen etc.) has yet been tried in man in such cases‡.

The clinical history of eight physicists irradiated in Los Alamos at the same time with small doses is interesting, in that only one of them showed mild but typical radiation sickness: nausea, little vomiting on the first day, a slight rise in temperature, prostration for a few days, and then no symptoms for a fortnight. During the third week partial epilation, marked fatigability persisting for several months and prolonged sterility, set in. There was complete recovery of fecundity after several years. Every irradiated person, even after only a small dose, should be kept under observation for a month, and physical activity should be restricted to a minimum.

\* Phlyctena on the third day, then loss of the skin of the hands and forearms, dermal necrosis and gangrene.

† Recent research emphasizes that the cells of the oligodendroglia of rabbits, rats and mice are very sensitive to radiations. The retinal rods, occasional neurones of the pyramidal lobe and of the olfactory region, and the sub-ependymal cells are also very radiosensitive (200 r)<sup>10, 11</sup>. The weight of the dried brains of mice increases by 4 to 14 per cent after a lethal or sublethal dose of x-rays. This is the only organ which reacts in this way; all others take up water<sup>13</sup>. The neuroblast, the embryonic nerve cell, is one of the most sensitive cells to ionizing radiations, a dose of 40 r is sufficient to destroy the neuroblasts of foetal mice<sup>12</sup>.

‡ SARACINO<sup>14</sup> claims to have obtained good results in leucopenia in irradiated cancer patients with intramuscular injections of 2.5 per cent sodium thymonucleate (5 to 10 cc per day).

## RADIATION HAZARDS OF THE POPULATION

### RADIATION HAZARDS OF THE POPULATION

The rapid increase in the number of establishments using atomic energy, and the increasingly widespread use both of radioactive isotopes and of radiographic examination, confront human radiobiology with a series of problems which can seldom be answered with precision in the present state of our knowledge. In most cases only cautious approximations are possible.

The hazards of work in atomic establishments and institutions where cyclotrons, radium, or large quantities of radioactive substances are used, are a calculated risk which must be accepted in the same way as the dangers inherent in obtaining coal or oil. So long as atomic energy is strictly controlled by competent men, only a few people will be exposed to danger; compared with the whole population the proportion is minute and negligible. If atomic weapons were to be used on a large scale in war this would, of course, no longer be true, for the genetic danger arises as soon as whole populations are exposed to irradiation of the whole body with doses of the order of 20 r repeated in each generation.

There are many problems, both in peace and in war. In peace time those whose occupation exposes them to doses far above the normal (see for example WILLIAMS<sup>28</sup>) must be protected as far as possible, but it is also an absolute duty to provide really efficient protection to other groups such as workers in nuclear research establishments (pure or applied research, and soon, presumably, industrial establishments), those who handle radium and isotopes, and radiologists. International commissions\* have fixed a 'maximum permissible dose' of 0.3 r per week, which was obtained by dividing the minimum dose which causes the mildest symptoms of radiation sickness by a large safety factor†. But 0.3 r per week

\* There are several permanent international commissions: the Joint Committee on Radiobiology, the International Commission on Radiological Protection, and the International Commission on Radiological Units. These Commissions met at Stockholm, September 15th to 20th 1952, and their proceedings were reported in *Acta radiologica*, 1954, **41**, fasc. 1. Reports on the activities of these Commissions are given at the International Congresses of Radiology. They are consultative bodies and cannot enforce the measures they recommend. In addition, radiobiologists (in the widest sense of the term, including physicists, physical chemists, cytologists, physiologists, biochemists and geneticists) meet annually in an informal Symposium (Aarhus 1953, Liège 1954).

† The strictly controlled application of these precautions has, however, not prevented the occurrence of some accidents (see above, p. 348). An unusually high incidence of cataract and an abnormal form of leucocyte have also been described recently in workers with cyclotrons.

The number of leucocytes is often considered as the most sensitive test; but much effort has been expended uselessly, in blood counts (see reference 33). The Stockholm conference has accepted the view that the dose above which blood counts

#### OBSERVATIONS IN HUMAN BEINGS

amounts to 400 r in 26 years (*i.e.* in the course of active sexual life), which is five times the dose which, according to geneticists, doubles the incidence of spontaneous mutations. The genetic danger is slight provided that only a few individuals are involved when it becomes unlikely that both man and wife were irradiated or that successive generations are similarly exposed.

With this dose of 0·3 r the danger to the individual from the carcinogenic action of radiations is practically nil. Increased incidence of cancer among radiologists (see LACASSAGNE<sup>23</sup>) has disappeared since the hazard was recognized and since means of protection have been devised.

Atomic research establishments are so arranged that the smallest possible quantity of radioactive material escapes into the air or into sewers. There is continuous supervision, and arrangements are made to bury the radioactive wastes in a concentrated and water-soluble form. LOUTIT<sup>24</sup> has calculated that if all the heat obtained from coal in Great Britain were replaced by energy from atomic fission the total activity (expressed as strontium 90) would be 8500 megacuries. If this were 1 per cent of the world production, and if this were evenly distributed in the water of the oceans, the concentration would be  $6 \times 10^{-7}$  microcuries per ccm, which is the permissible concentration for drinking water. As a matter of fact the oceans already contain 300,000 megacuries in the form of radioactive potassium. It would, however, be necessary to avoid the formation, even for a short time, of high concentrations in certain seas or currents. Unfortunately it takes a long time for radioactive substances discharged into the sea, or formed in it, to become evenly distributed.

Some isotopes must be watched more carefully, because they accumulate in certain tissues in man, for instance iodine in the thyroid and strontium and barium in bone. Certain isotopes scattered on pastures may become concentrated in animals used by man as food. One of the most dangerous isotopes in this respect is <sup>14</sup>C, which has a long life (3800 years) and which in the form of CO<sub>2</sub> is directly incorporated by the human and animal body, and which may reach it indirectly through the plants which use it as a source of carbon for all their syntheses.

But, as MULLER<sup>25</sup> rightly states, the danger is much greater for the population of so-called civilized countries, because of the great increase in radiological examination. Physicians and surgeons must

---

are advisable is 0·12 r per week; below this level it is doubtful whether any significant information can be obtained from such counts. In rats the number of lymphocytes does not change even after a daily dose of 2·5 r for 600 days<sup>34</sup>.

#### RADIATION HAZARDS OF THE POPULATION

be reminded that a complete radiosscopic and radiographic examination of the stomach subjects the patient to a dose of about 50 r and examination of the lungs exposes to a dose of 8 to 10 r. These exposures should be kept to a minimum by radiographers\*. In some countries the following reprehensible practices are becoming wide-spread: (i) irradiation of the testicles of young men with doses up to 500 r to produce temporary sterility; (ii) irradiation of the ovaries of women to stimulate fertility; and (iii) radiography during pregnancy, which has the two-fold disadvantage of irradiating both the ovaries of the mother and of the forerunners of the gametes of the foetus.

According to all the evidence direct irradiation of the gonads should be carried out only in exceptional circumstances during the reproductive years, when no other method of diagnosis can provide information of the same value. Since the future will probably bring a continuous increase in the basic radioactivity of our planet, we share MULLER's wish<sup>25</sup> that physicians and surgeons would prescribe radiosscopic examination only when it is really necessary. Deep radiotherapy in cancer or in the aged does not constitute a genetic risk, and radiotherapists are fully justified in applying larger doses of ionizing radiations even to the gonads.

It is hardly necessary to emphasize that governments should prohibit the use of badly designed television sets which allow ionizing radiations to escape from the tube, and of the x-ray apparatus used in shoe shops to irradiate the feet of customers—often children—to show the fit of shoes. It should be the duty of all physicians, with the support of strict legislation, in their daily practice, and of all hygienists to suppress unnecessary exposure to ionizing radiation. Similar considerations apply to the use of radiomimetic chemicals (see Chapter 7).

The natural radioactivity of the soil, of building materials and of foods, and the radiations of cosmic origin are extremely weak but not negligible. It is estimated that the annual dose of ionizing radiation received by man is 0·1 r. In some regions, such as Scandinavia and Scotland, where primitive geological formations, such as granite, crop out, this natural radiation is much greater, but the people who have lived there for thousands of years have not shown any more genetic disturbances than any other civilized peoples.

---

\* In the neighbourhood of New York among two hundred operators of diagnostic x-ray apparatus not one knew the output of their instruments, which were found to vary widely<sup>26, 27</sup>.

#### OBSERVATIONS IN HUMAN BEINGS

The distinctly pessimistic attitude of the geneticists<sup>25, 29</sup> concerning the recently introduced radiation hazards is not shared by all radiobiologists, but in our opinion no one has the right to neglect MULLER's<sup>25</sup> close reasoning, or to evade the problem in view of our limited quantitative knowledge concerning genetics. Geneticists base their views on the following experimentally established facts : (i) The effect of ionizing radiations on the genes is strictly cumulative; there is no restoration of genetic damage. A dose of 20 r is equally dangerous whether it is given in a single dose or in the course of several years starting at the time of conception (that is, including the foetal period); (ii) mutations are nearly always unfavourable to the race; (iii) natural selection plays a progressively less important part in the human race as fecundity diminishes, and hygienic and medical care becomes more efficient. This means that the genetic danger is much greater among the civilized peoples. For instance, the populations of the high plateaux of the Andes and Tibet show no signs of degeneration, though each generation receives about 5 r more cosmic radiation than the bulk of other populations. The higher fecundity and mortality of these primitive peoples ensure a certain degree of genetic adaptation.

MULLER<sup>25</sup> has calculated that if the incidence of mutations is the same in man as in mice a dose of 80 r would probably double the frequency of spontaneous mutations, would cause 'genetic death' of 2 individuals out of 5, and would considerably impair the health of the community if it were repeated from generation to generation. This could be attained if the radioactivity of the soil, air and food, together with medical or accidental irradiation, provided an average dose of 4 to 2 r per annum for the first 20 to 40 years of life. Muller thinks that a quarter of this dose, that is 20 r, is about the minimum dangerous dose for populations living in fairly primitive conditions, and should in no case be exceeded. This genetic danger may be one of the limiting factors in the development of nuclear energy.

It must be emphasized that all these calculations are based on two hypotheses which are probably true, but cannot be proved; firstly, that the frequency of spontaneous mutations is the same in human beings as in mice, and secondly, that the number of genes in man is about 15,000.

*Results of the atomic explosions in Japan*—The exposed population has been the subject of many well conducted investigations and statistical studies. A mixed team of Japanese and Americans has been observing survivors of the 1945 bombing and their offspring. The first reports<sup>15, 16</sup> agree on the following points:

- (i) There is an increased incidence (which cannot be precisely

## RADIATION HAZARDS OF THE POPULATION

determined) of leukaemia in people who were within 2 km of the point where the atom bomb burst\*.

(ii) Ten per cent of the survivors who were within 1·2 km of the bomb at Hiroshima show more or less marked changes in the crystalline lens. This cataract seems to be mainly due to neutrons†.

(iii) Growth and development appear to be the same in irradiated children, even in those irradiated *in utero*, as in control children who moved into the two cities after 1945.

The genetic changes produced are the most difficult to study and to interpret; even the figures given in statistical studies are contested. The problem is of the utmost importance as it is to be hoped that such an experiment will never be repeated and the maximum amount of information should therefore be obtained. In genetics, the transfer of results from animals to man is hazardous, and experience in Japan offers a unique opportunity to discover how sensitive man is to the mutagenic effect of ionizing radiations.

Geneticists and statisticians have set out a vast programme of research. Groups of irradiated parents have been selected, and very strict controls established. More than 50,000 infants have been examined, and are being regularly followed up. Statisticians would prefer numbers twice as large, but these are not available. BUCHER<sup>15</sup> states that 'it has been found that detectable anomalies of a genetic character have appeared in 1·18 per cent of the offspring of parents from the control population, while among the offspring of parents who showed evidence of radiation injury, the corresponding figure is 1·40 per cent'. This difference is small, but might have proved significant had not the more recent figures of NEEL *et al.*<sup>16</sup> led to the conclusion that there is no statistically significant difference between

---

\* In the past an increase in the frequency of leukaemia amongst radiologists has been well established, although this is no longer the case as more adequate precautions are now taken.

† In fact, the relative biological efficiency (RBE) is very great for neutron irradiation of the lens and gonads (see p 75). Only fast neutrons seem to cause opacities in the lens in small or fractionated doses. This observation is of the highest importance in work with cyclotrons and linear accelerators<sup>17</sup>. It seems to be very difficult to prevent these opacities from progressing to definite cataract. Several investigations show that the biochemical lesion is produced very early<sup>18-21</sup>. After irradiation the epithelium of the lens behaves in the same way as any other germinative epithelium. There is arrest of mitosis for 3 to 4 days, visible after only half an hour, followed by recovery of mitotic activity and over-compensation, fragmentation of the nuclei during the two hours following irradiation, and vacuole formation. The permeability of the lens is increased. Cysteine injected intravenously partly protects the lens from the action of x-rays. Unpublished preliminary observations by TANSLEY<sup>22</sup> suggest that cysteamine also has this protective effect.

#### OBSERVATIONS IN HUMAN BEINGS

the children of irradiated and non-irradiated parents\*. However, the optimistic conclusion that man is fortunately not very sensitive to the mutagenic action of ionizing radiations is not justifiable; firstly, because that what is true of the Japanese is not necessarily true of all other races, and, secondly, because the second, or even the third, generation must be awaited before it can be certain that the atomic bombs have not caused a genetic disaster. Unfortunately, future statisticians will probably not find enough children in the relevant groups, which will become more and more complicated as time goes on.

#### REFERENCES

- 1 HERVE, A., *J. belge Radiol.*, 1952, **35**, 655
- 2 ELLIS, F., *J. Fac. Radiol., Lond.*, 1951, **3**, 207
- 3 BROWN, W. M. COURT, *Brit. med. J.*, 1953, **1**, 802
- 4 BROWN, W. M. COURT and MAHLER, R. F., *Science*, 1953, **118**, 271
- 5 BROWN, W. M. COURT and MAHLER, R. F., *Proc. R. Soc. Med.*, 1953, **46**, 245; *J. Fac. Radiol., Lond.*, 1954, **5**, 200
- 6 BACQ, Z. M. and HERVE, A., *Abstr. 7th int. Congr. Radiol.*, Copenhagen, p. 133
- 7 HERVE, *Rev. Méd. Liège*, 1952, **7**, 276
- 8 BACQ, Z. M., *Bull. Acad. Méd. Belg.*, 1953, 7th series, **18**, 426
- 9 HEMPELMANN, L. H., LISCO, H. and HOFFMAN, J. G., *Ann. intern. Med.*, 1952, **36**, 279
- 10 GEREBTZOFF, M. A. and HERVE, A., *C. R. Soc. Biol.*, 1949, **143**, 880
- 11 HICKS, S. P. and MONTGOMERY, P. O'B., *Proc. Soc. exp. Biol. Med.*, 1952, **80**, 15
- 12 HICKS, S. P., *Proc. Ass. Res. nerv. ment. Dis.*, Williams and Wilkins, Baltimore, 1953, **32**, 439
- 13 RUGH, R., *Nucleonics*, 1954, **12**, No. 1, 28
- 14 SARACINO, R., *Sem. médicale*, 26 May 1952, p. 411
- 15 BUCHER, J. C., *Nucleonics*, 1952, **10**, No. 9, p. 18
- 16 NEEL, J. W. et al., *Science*, 1953, **118**, 537
- 17 EVANS, T. C., reported at *Aarhus Conf.*, 1953
- 18 SALLMANN, L. VON, *Trans. Amer. ophth. Soc.*, 1952, **49**, 391
- 19 SALLMANN, L. VON, *Arch. ophth. Amer. med. Ass.*, 1952, **47**, 305
- 20 SALLMANN, L. VON and LOCKE, B. D., *ibid.*, 1951, **45**, 431
- 21 SALLMANN, L. VON, DISCHE, Z., EHRlich, G. and MUÑOZ, C., *Proc. Ass. Res. Ophth.*, 9th Meet, 1950, p. 95
- 22 TANSLEY, C., personal communication, 1953
- 23 LACASSAGNE, A., *Les cancers produits par les rayonnements électromagnétiques, Les cancers produits par les rayonnements corpusculaires*, Hermann, Paris, 1945

\* The sex-ratio at birth is changed at Nagasaki, but not at Hiroshima. Stillbirths seem to be slightly more frequent in the irradiated population of both cities. There is hardly any difference in the weight of infants at birth<sup>16</sup>.

#### REFERENCES

- <sup>24</sup> LOUTIT, J. F., *Nature, Lond.*, 1954, **173**, 621
- <sup>25</sup> MULLER, H. J., *Acta Radiol.*, 1954, **41**, 5
- <sup>26</sup> SONNENBLICK, B. P., *Genetics*, 1952, **37**, 627
- <sup>27</sup> SONNENBLICK, B. P., LEVINSON, L. J., FURST, N. J. and KOCH, J.,  
*J. Newark Beth Israel Hosp.*, 1952, **2**, 153
- <sup>28</sup> WILLIAMS, E. K., *Acta Radiol.*, 1954, **41**, 21
- <sup>29</sup> EHRENBURG, L. and GUSTAFSSON, A., *ibid.*, 1954, **41**, 101
- <sup>30</sup> RAMIOUL, H., 1954, in press
- <sup>31</sup> VAN DE BERG, F. and VAN DE BERG, L., 1954, in press
- <sup>32</sup> *Effect of Atomic Weapons*, U.S. Dep. Defence and U.S. Atom. Energy Comm., New York, 1950
- <sup>33</sup> WILLIAMS, E. K., *Acta Radiol.*, 1954, **41**, 21
- <sup>34</sup> LANGENDORFF, H., *Strahlentherapie*, 1953, **90**, 408

## POSTSCRIPT

WE believe that it is possible even at the present stage of radiobiological research to formulate a coherent, if necessarily incomplete, picture of the phenomena.

The observations, that almost all biological effects brought about by radiations of low specific ionization are reduced (i) by anoxia and (ii) by the presence of a number of chemical substances, may become laws of general radiobiology. The findings can best be explained by the intervention of free radicals formed by the radiations in the aqueous phase of the organism. In view of the high local concentration of certain components in the cell (*e.g.* nucleoproteins in the chromosomes) direct energy absorption must also play a part in the production of chemical changes leading to the observed biological effects. Such direct action is now known to be influenced by extraneous factors (*i.e.* protection is possible) and need no longer be regarded as an unalterable event determined solely by the irradiation.

The primary chemical changes produced during the irradiation probably cannot be reversed after irradiation, and the chemical protectors act in mammals only if given before irradiation, when they reduce the number of primary chemical changes produced. The subsequent biochemical lesions cannot yet be defined exactly, but a large body of evidence shows that they are various and not necessarily the same in different species. These biochemical lesions are responsible for the observed physiological, histological and some of the genetical changes, all of which appear more quickly if the general metabolism is high. A possible interpretation is that the chemical energy at the disposal of the cell is limited after irradiation by the breakdown of the sensitive equilibrium of the intracellular structures resulting in the decreased activity of enzyme systems observed in the *later* stages after irradiation. In addition to these effects, the production of chromosome abnormalities, which is responsible for many of the permanent genetic changes, may also interfere with the efficient division of the cell by mechanically ob-

#### POSTSCRIPT

structing mitosis. In all these considerations it is of the greatest importance to bear in mind the possible restitution of the biological lesion by restoration processes.

\* \* \*

Based on these concepts four applications of chemotherapy in the domain of human radiobiology (in particular radiotherapy) can be predicted and have to some extent already been achieved:

(i) To diminish the effects of ionizing radiations by administration of a protector before irradiation. Cysteamine (Z. M. Bacq), a physiological substance, and its disulphide derivative, cystamine, fulfil this requirement and in addition also alleviate the symptoms of mild forms of radiation sickness;

(ii) to increase locally, in neoplastic tissues, the effectiveness of ionizing radiations by the administration of a sensitizer which concentrates in the tumours. In this field the findings of J. S. Mitchell are full of promise;

(iii) to reverse the principal chemical and biochemical changes by injecting within a short time (*e.g.* 30 minutes) after irradiation a substance which restitutes the damaged molecules, in the same way as dimercapto-propanol (BAL of R. A. Peters) causes regression of the lesions from lewisite poisoning. Current research provides no clue since we have insufficient knowledge of the biochemical lesions resulting in radiation damage, and there is little hope of finding a single powerful antidote; and

(iv) to apply after irradiation a factor which assists regeneration of the haemopoietic organs. The existence of such a factor seems certain and much is known concerning the organs in which it is localized and consequently we have every right to hope that it will be available within a few years.

Radiobiology has been transformed in the last ten years, during which the emphasis of research has moved away from the sterile pursuit of purely anatomical observations. The application of radiobiological research to the solution of many important practical problems seems hopeful and the future is full of promise.



## NAME INDEX

Italicized page numbers refer to those where the author is referred to by reference number only

- Abderhalden, R., 247  
Abel, E., 102, 212  
Ackermann, I. B., *208*  
Adamson, D. M., *241*, 257  
Aebersold, P. C., 75  
Albaum, H. G., *243*  
Alder, M. G., 89, 91  
Alderman, I. M., *288*  
Alexander, P., *49*, 62, 63, 66, *84*, 86, 89,  
97, *105*, *106*, *110*, *111*, *122*, *125*, *130*,  
*142*, *144*, *145*, *146*, *150*, *153*, *178*, *193*,  
*195*, *196*, *203*, *204*, *290*, *292*, *293*, *294*,  
*301*, *302*, *303*, *305*, *307*, *308*, *311*, *313*,  
*315*, *321*  
Alfert, M., *179*  
Allen, J. G., *191*, 283  
Allen, O. D., *84*, 86, 87  
Alper, T., *96*, *107*, *108*, *111*, *113*, *149*,  
*218*, *234*  
Altland, P. D., *341*  
Altman, K. I., *186*, 245, 247, 248, 254  
Ancel, P., *184*, *246*  
Anderson, E. H., *67*, *71*, *210*, *212*  
Anderson, R. S., *136*, *141*  
Anger, H. O., *29*  
Appelmans, F., *186*  
Appleyard, R., *141*  
Arnow, L. E., *137*, *138*  
Ashwell, G., *187*, *242*, *243*, *251*  
Astbury, W. T., *134*, *143*  
Auerbach, C., *190*, *200*, *201*  
Aurand, B., *220*  
Austin, M. E., *253*  
Avery, O. T., *177*  
Back, A., *171*, *250*, *251*  
Bacq, Z. M., *110*, *111*, *182*, *183*, *185*, *186*,  
*192*, *196*, *197*, *202*, *215*, *222*, *223*, *225*,  
*234*, *235*, *236*, *237*, *241*, *248*, *249*, *252*,  
*267*, *270*, *275*, *283*, *288*, *290*, *291*, *292*,  
*293*, *294*, *295*, *296*, *297*, *298*, *299*, *300*,  
*301*, *302*, *303*, *304*, *305*, *306*, *307*, *308*,  
*311*, *312*, *313*, *314*, *316*, *317*, *318*, *319*,  
*321*, *334*, *345*  
Baddiley, J., *295*  
Bair, W. G., *247*  
Baker, W. K., *67*, *71*, *75*, *210*  
Baldwin, T. N., *152*  
Ballin, J. C., *246*, *247*  
Balwit, J. S., *124*, *125*  
Bang, J., *145*  
Barb, W. G., *96*  
Bardwell, D. C., *38*  
Barnes, D. W. H., *331*, *336*  
Barnum, C. P., *180*  
Barron, E. S. G., *72*, *113*, *114*, *136*, *137*,  
*138*, *139*, *140*, *141*, *152*, *183*, *233*, *240*,  
*247*  
Bartingdale, G. W. R., *103*  
Bates, J. C., *19*  
Beatty, A. V., *71*  
Beatty, C. H., *228*, *241*  
Beaullieu, M. M., *233*  
Beaumariage, M. L., *275*, *318*  
Bekkum, D. W. van, *243*  
Bellack, S., *304*  
Bennet, L. R., *216*, *291*  
Berg, F. van de, *346*  
Berg, L. van de, *298*, *346*  
Berger, H., *292*  
Bergonié, J., *156*  
Bernard, J., *270*, *298*, *300*, *321*  
Bernas, A., *39*, *81*, *103*  
Bertani, G., *191*  
Berthel, J., *186*  
Berthel, L., *186*  
Bethe, H., *13*, *30*  
Betz, H., *229*, *271*, *276*, *291*, *306*, *316*,  
*318*, *328*, *331*  
Bhatia, D. S., *112*  
Bieseck, J. J., *193*  
Bigelow, R. R., *281*  
Bihan, H. Le, *293*, *295*, *301*, *308*, *316*, *319*  
Billamy, W. D., *152*  
Billen, D., *68*, *69*, *265*  
Binet, L., *191*  
Bingemann, M. E., *301*  
Bird, M. J., *193*

## INDEX

- Blackford, M. E., 306, 310  
 Blackwood, O., 66  
 Blair, H. A., 332, 333  
 Blavier, J., 293, 295, 301, 308  
 Blinks, L. R., 224  
 Bloch-Frankenthal, L., 171, 250, 251  
 Block, M. H., 191  
 Bloom, W., 171  
 Blum, H. F., 263, 264  
 Boag, J. W., 107, 108, 272  
 Bohr, Niels, 13  
 Bohrensyayn, Ch., 298  
 Boivin, A., 177  
 Bond, V. P., 272, 331, 334, 336, 337, 338,  
     339, 340, 343  
 Bonet-Maury, P., 88, 220, 290, 291, 303  
 Bothwell, J., 232  
 Botkin, A. L., 253  
 Bowers, J. L., 274  
 Boyland, E., 115, 191, 203, 292  
 Brachet, J., 179, 180, 243, 336, 342  
 Bragg, W. M., 17, 80  
 Brecher, G., 284, 293, 316, 318, 333, 334  
 Breger, I. A., 41  
 Brennan, J. T., 75  
 Brien, P., 223  
 Brohult, S., 63, 128, 135, 136, 137  
 Brook, R. E., 151, 152  
 Brown, D. M., 143, 304  
 Brown, M. B., 330, 336, 339  
 Brown, W. M. Court, 345  
 Brownscombe, E. R., 81, 101, 107, 110,  
     111  
 Brues, A. M., 16, 238, 240, 290, 293, 316,  
     330, 332, 333, 336  
 Bruyn, P. P. H., 333  
 Bryson, V., 201  
 Buckley, S. M., 193  
 Buddington, W. G., 229  
 Bueche, A. M., 124, 125  
 Buffa, P., 232  
 Bugher, J. C., 352, 353  
 Bunce, B. H., 60, 143  
 Burchenal, J. H., 192, 193  
 Burg, C., 234, 235, 237  
 Burke, A. W., Jr., 293, 303  
 Burn, J. H., 239, 256, 280  
 Burnett, W. T., 210, 293, 303  
 Burnett, W. T., Jr., 309  
 Burton, M., 35, 39, 42, 52, 84, 104, 127  
 Burton, V. L., 41  
 Bush, H., 232  
 Butler, C. L., 153, 257  
 Butler, J. A. V., 148, 149, 150, 203, 204  
 Butler, G. C., 147, 148, 149, 150  
 Buu-Hoi, N. P., 301  
 Buzzell, A., 60  
 Byers, S. O., 256  
 Caldwell, P. G., 178  
 Calgan, J., 284  
 Cameron, A. F., 80  
 Camien, M. N., 225  
 Campbell, J. A., 214  
 Carter, R. F., 75  
 Carttar, M. S., 237, 242, 243  
 Casarett, G. W., 245, 254  
 Casarini, A., 197, 198  
 Caspersson, T., 179  
 Catcheside, D. G., 67, 69  
 Cauwenberg, H. van, 270, 298  
 Cerf, R., 144  
 Chambers, F. W., Jr., 271  
 Chambers, F. W., 291, 293, 316  
 Chantrenne, H., 180  
 Chanutin, A., 186, 187, 203, 238, 251  
 Chapiro, A., 39  
 Chapman, W. H., 271, 291, 293, 316, 318  
 Chargraff, E., 142, 143  
 Charipper, H. A., 271, 276  
 Charlesby, A., 49, 62, 63, 66, 122, 123,  
     124, 125, 126, 127, 128, 132, 135, 136,  
     313  
 Charlier, R., 301, 310  
 Chase, H. B., 191  
 Chastain, S. M., 216, 291  
 Cheng, A. L. S., 301  
 Chevallier, A., 234, 236, 237  
 Chévremont, S. and M., 298  
 Christensen, H. N., 225  
 Christensen, W. R., 238, 287  
 Christian, E. J. B., 238  
 Clark, J. W., 310  
 Clarke, G., 253  
 Claude, A., 157, 185, 336  
 Cleland, G. H., 191  
 Cochran, K. W., 232, 233, 242  
 Cohen, P. P., 175  
 Cole, L. J., 301, 303, 312, 331, 334, 336,  
     337, 338, 339, 340  
 Collison, E., 82, 96, 102, 116, 137, 140  
 Comhaire, S., 72  
 Comsa, J., 229  
 Conard, R. A., 256

## INDEX

- Conger, A. D., 70, 75, 97, 106, 108, 111, 169, 210, 212, 216  
Congdon, C. C., 336, 340  
Congdon, Ch., 336  
Conway, B. E., 148, 149, 150, 203, 204  
Cooke, A. R., 235  
Cool, W. S., 341, 342  
Coon, J. M., 303  
Cormack, A., 25, 26, 27  
Corson, M., 115  
Costello, M. J., 242  
Coulson, C. A., 65, 67, 68, 102  
Coulter, M. P., 231, 242, 274, 331  
Cour, L. F. La, 163, 179  
Cousin, C., 39  
Crabtree, H. G., 209, 292  
Cramer, W., 209  
Crampton, C. F., 142  
Crick, F. H., 143, 144  
Cronkite, E. P., 271, 272, 276, 282, 284, 285, 291, 293, 316, 318, 333, 334  
Crowther, J. A., 54, 66, 116, 117  
Cudkowicz, G., 235  
Cuendet, A., 271, 272, 281  
Curie, P., 37, 79
- Dainton, F. S., 15, 81, 82, 84, 90, 96, 100, 102, 103, 107, 108, 111, 113, 116, 137, 140, 150  
Dale, W. M., 24, 47, 48, 49, 55, 57, 64, 83, 85, 93, 95, 97, 98, 112, 113, 114, 139, 140, 141, 150, 312  
Dallemande, M. J., 233  
D'Amato, F., 169, 292  
D'Ancona, S., 226  
D'Ancona, U., 226  
Daniel, G. E., 223, 224  
Danielli, J. F., 157, 191  
Daniels, D. S., 70  
Daniels, F., 43  
Daniels, M., 149  
Darlington, C. D., 157, 160, 163, 193  
Davidson, J. N., 179, 180  
Davies, J. V., 112, 113  
Davison, S., 19  
Dawson, I. M., 180  
Day, M. J., 43  
Debierne, A., 79  
Debley, V., 288  
Dechamps, G., 293, 295, 297, 308, 316, 318, 319  
Delaunay, A., 177  
Deltour, G., 270, 298, 300, 321  
Demerec, M., 191  
Dempster, E. R., 75  
Dempster, W. J., 272  
Denfle, J., 199  
Desaive, P., 288, 318  
Detrick, L. E., 288  
Deuel, H. J., Jr., 301  
Devik, F., 310, 320  
Dewhurst, H. A., 102  
Deysson, G., 298  
Dickey, H. H., 191  
Dickman, S., 240  
Dillard, G. H. L., 284  
Dittrich, W., 116  
Dobrovolskaia-Zavadskiaia, N., 232  
Doernbach, E. F., 229  
Doherty, G. D., 297, 313  
Dooley, R., 342  
Doty, P., 60, 143  
Doudney, C. O., 305  
Doull, J., 184, 232, 233, 242  
Dounce, A. L., 178  
Dowdy, A. H., 216, 271, 291, 300, 308  
Doyle, B., 54, 60, 62, 63  
Drew, R., 60, 144  
Dreyfus, G., 244, 253  
Duane, W., 80  
Dubois, K. P., 184, 232, 233, 242, 243  
Dubouloz, P., 234  
Duchateau, G., 225  
Duffy, B. J., 277  
Dumas, J., 234  
Dunjic, A., 320, 332, 333, 334  
Duplan, J. F., 301  
Duryee, W. R., 170, 183, 184  
Dustin, P., 190, 234, 284  
Dustin, P., Jr., 191  
Duve, Chr. de, 186  
Dwyer, P., 298  
Ebert, M., 70, 75, 96, 97, 106, 107, 108, 111, 113, 216  
Eddy, C. E., 72  
Edelmann, A., 271, 272, 276  
Ehrenberg, L., 66, 75, 352  
Eichel, H., 233, 256, 266, 305  
Eldjarn, L., 295, 296, 318  
Eldredge, J. H., 331, 332, 333, 334  
Ellinger, F., 237, 246, 272  
Ellis, F., 344

## INDEX

- Ellis, M. E., 301, 303, 312, 331, 334, 336, 337, 338, 340  
 Elmore, D. T., 195  
 Elson, L. A., 202, 238, 287  
 Eltzholz, D. C., 271, 291, 293, 316  
 Ely, J. O., 230  
 Emerson, D. M., 283  
 Entenman, C., 180, 239, 241, 253  
 Ephrussi-Taylor, H., 177, 218, 226  
 Errera, J., 42, 115  
 Errera, M. C. R., 152, 149, 152, 229  
 Essex, H., 38  
 Euler, H. von, 170, 247, 253, 256, 257  
 Euler, U. S. von, 303  
 Evans, T. C., 75, 223, 224, 253, 353  
 Eyring, H., 30, 36, 38, 39, 89, 91  
 Fahmy, O. G., 193  
 Failla, G., 72, 224  
 Fairbrother, 116  
 Fairchild, L. M., 169, 210  
 Fano, U., 75  
 Farr, R. S., 333  
 Feinstein, R. N., 153, 246, 247, 257  
 Felix, K., 145, 177  
 Fenn, W. O., 298  
 Figge, F. H., 304  
 Finkelstein, P., 136, 137, 138  
 Fiquet, F., 81, 103  
 Firke, J., 72, 202, 203  
 Fisher, H., 145  
 Fisher, M. A., 242, 295, 296, 297, 298  
 Fisher, P., 228, 230, 231, 241, 270, 275, 291, 293, 298, 299, 300, 301, 305, 308, 313, 316, 318, 319, 320, 328  
 Fishler, M. C., 331, 334, 336, 337, 338, 339, 340  
 Flint, J., 191  
 Flood, V., 72, 141, 233, 240  
 Florkin, M., 225  
 Florsheim, W., 229  
 Flude, D., 60, 144  
 Fondaraï, J., 234  
 Ford, C. E., 198  
 Forro, F., 60  
 Forssberg, A., 140, 181 220, 222, 228, 230, 245, 253, 254, 255, 281, 310, 311, 315  
 Forster, T., 33  
 Fox, M., 97, 105, 106, 130, 131, 132, 133, 134, 150, 195, 203, 305, 313, 314, 315  
 Franchi, M., 291  
 Francis, D. S., 72  
 Franck, J., 33, 40, 94  
 Frederic, J., 241  
 Fricke, H., 52, 81, 84, 87, 90, 92, 97, 101, 107, 108, 110, 111, 112, 137, 141  
 Friedman, J., 272  
 Fritz-Niggli, H., 116  
 Froehlich, H., 86  
 Fruhling, L., 316, 318  
 Furchner, J. E., 333  
 Furth, F. W., 231, 274, 281, 331  
 Gallico, E., 292  
 Galton, D. A. G., 202  
 Gamow, G., 178  
 Gardner, W. V., 304  
 Gaston, E. O., 330, 331, 332, 337  
 Gasvoda, B., 72  
 Gates, E., 253, 284  
 Gay, H., 201  
 Gelin, O. E., 72  
 Gennes, L., 298  
 Gerebtzoff, M. A., 316, 319, 328  
 Gerschmann, R., 298  
 Gershon-Cohen, J., 332  
 Chormley, J. A., 19  
 Gilbert, C. W., 112, 113  
 Gilbert, D. L., 298  
 Gilbert, L. A., 203, 204  
 Giles, N. H., 71, 75, 211  
 Gjessing, E. C., 203, 238  
 Goblet, J., 298  
 Godeaux, J., 105  
 Goldacre, R. J., 205  
 Goldberg, B., 191  
 Goldblith, S. A., 19, 97, 115  
 Goldin, A., 191  
 Gompel, C., 234  
 Gordon, A. S., 271, 276  
 Gordon, L. E., 276  
 Gordon, S., 42, 51  
 Goutier, R., 186  
 Graffeo, A. J., 272  
 Graham, A. B., 272  
 Graham, J. B., 272  
 Graham, R. M., 272  
 Grampa, G., 191  
 Gray, J. L., 215, 303  
 Gray, L. H., 11, 13, 16, 17, 18, 23, 24, 25, 26, 30, 47, 48, 70, 71, 73, 74, 75, 83, 85, 93, 94, 95, 96, 97, 98, 106, 107, 108, 111, 113, 117, 140, 171, 184, 216, 217, 258, 304, 336

## INDEX

- Greenstein, J. P., 142, 144, 146, 148, 149, 150, 151, 203  
 Grenan, M. M., 184, 229  
 Griffith, F., 177  
 Griffith, J. Q., Jr., 332  
 Grimaldi, A. J., 231, 235, 239  
 Grinnan, E. L., 144, 150  
 Gros, C., 183, 229, 318  
 Grosch, D. S., 223  
 Gulland, J. M., 195  
 Gustafson, A., 66, 75, 169, 292, 352  
 Haas, F. L., 292  
 Haddow, A., 159, 191, 192, 193, 202, 205  
 Haenisch, G. F., 264  
 Hahn, L., 170, 247, 253  
 Hahn, N. J., 276  
 Haines, R. B., 65, 67, 68  
 Haissinsky, M., 89, 101, 102, 103  
 Hajdukovic, S., 216  
 Haley, T. H., 234, 280, 288  
 Haley, T. J., 300, 308  
 Hall, B. V., 293  
 Hall, J. J., 151, 152  
 Halle, E. S. von, 71, 75  
 Halpern, B. N., 271, 272, 281  
 Halvorsen, H., 229, 253  
 Hammerling, J., 180  
 Harris, E. B., 191, 287  
 Harris, P. S., 75  
 Harris, R. J. C., 191  
 Hart, E. J., 52, 84, 87, 89, 90, 92, 97, 109, 111, 112  
 Hassett, C. C., 223  
 Haurowitz, F., 177, 203, 206  
 Hauschildt, J. D., 239  
 Hawn, Z., 176  
 Heitler, W., 6, 11, 12  
 Heller, J. H., 266  
 Hempelmann, L. H., 106, 348  
 Henderson, M. E., 225  
 Hendley, D. D., 257  
 Hendry, J. A., 193  
 Henri, V., 42, 115  
 Henshaw, P. S., 72, 224  
 Herbert, P., 230  
 Hermel, M. B., 332  
 Herve, A., 183, 185, 186, 202, 215, 216, 222, 223, 225, 235, 248, 249, 252, 281, 283, 284, 288, 290, 291, 292, 293, 294, 295, 297, 298, 299, 300, 301, 302, 303, Herve, A.—*cont.*  
                   304, 305, 306, 307, 308, 311, 312, 313, 316, 317, 318, 319, 321, 328, 333, 334, 344, 345  
                   Heuse, O., 220  
                   Heusghem, C., 234, 236, 237, 241, 269, 270, 298, 308, 328  
                   Hevesy, G., 176, 230, 233, 244, 245, 253, 254, 257, 281  
                   Hewitt, H. B., 66  
                   Hick, S. P., 348  
                   Hickman, J., 242, 243, 251  
                   Hueger, I., 190, 199  
                   Hill, R., 215  
                   Hinshelwood, C., 178  
                   Hirschfelder, J. O., 30, 36, 38  
                   Hitch, S. F., 132, 142, 144, 150  
                   Hochnadel, C. J., 19, 89, 90  
                   Hodge, H. C., 186  
                   Hodges, G. R. V., 308  
                   Hoffman, J. G., 348  
                   Hogboon, G. H., 185, 336  
                   Hollaender, A., 66, 67, 68, 69, 71, 72, 75, 146, 148, 149, 150, 151, 203, 210, 211, 213, 264, 265, 290, 293, 294, 300, 301, 303, 305, 308, 309, 310, 312  
                   Holmes, B., 137, 140  
                   Holthusen, H., 209, 264  
                   Homer, R. F., 193  
                   Honig, R. E., 41  
                   Horgan, V. J., 234  
                   Hornsey, S., 70, 75, 97, 106, 108, 111, 216  
                   Horowitz, N. H., 200  
                   Howard, A., 181  
                   Howland, J. W., 231, 239, 243  
                   Huber, W., 115, 116  
                   Hudson, R. F., 135  
                   Hursh, B., 253, 301  
                   Huseby, R. A., 180  
                   Hustrulid, A., 82  
                   Hutchinson, F., 60  
                   Ingram, M., 231, 239, 243, 331  
                   Ito, H., 257  
                   Jackson, E., 229, 310  
                   Jacobs, G. J., 284  
                   Jacobson, L. O., 191, 330, 331, 332, 333, 334, 337  
                   Jaffé, G., 85  
                   Jeener, R., 180  
                   Jeffreys, C., 60

## INDEX

- Jennings, F. L., 288  
 Jensen, E. V., 203  
 Jensen, H., 215, 253, 303  
 John, E. S., 275  
 Johns, B., 25, 26, 27  
 Johnson, E. R., 102  
 Jollcs, B., 329, 331  
 Jones, H. B., 245  
 Jordan, D. O., 195
- Kahn, J. B., Jr., 304  
 Kailan, A., 80, 81, 111  
 Kan, B., 19  
 Kaplan, H. S., 288, 304, 318, 330, 331, 332, 333, 336, 339  
 Karel, M., 19  
 Kaufmann, B. P., 201  
 Kayser, C., 216, 218  
 Kelly, L. S., 180, 253  
 Kennaway, E. L., 190  
 Kenokh, M. A., 151  
 King, E. D., 304  
 Kirby-Smith, J. S., 70  
 Kirnbaum, M., 80  
 Kirschner, L. B., 229  
 Klein, G., 181, 228, 255  
 Klein, O., 8  
 Kluyskens, P., 298  
 Knox, W. E., 246  
 Koch, R., 272, 304, 330, 331  
 Koch, H. J., 280  
 Koenig, V. L., 136, 147  
 Kohn, H. T., 231, 235, 238, 239, 276  
 Koller, P. C., 165, 172, 179, 191, 193, 197, 198, 202  
 Kolthoff, I. M., 100  
 Kon, G. A. R., 191, 192  
 Kordik, P., 239, 256, 280  
 Kotval, J. P., 71, 75  
 Kowlessar, O. D., 186  
 Krebs, A. T., 304  
 Krekels, A., 145  
 Kryder, G. D., 301  
 Kunckel, H. O., 251
- Lacassagne, A., 216, 276, 290, 301, 303, 318, 350  
 Lajta, L. G., 256  
 Lamarque, J. P., 183, 263  
 Lambert, G., 266, 294, 331, 332, 333  
 Lambert, J., 72, 248, 249  
 Lambert, S., 298
- Lamerton, L. F., 17, 191, 238, 287, 294, 316, 332  
 Lancker, J. van, 332, 333, 334  
 Landing, B. D., 191  
 Landtsheer, L. De, 228, 241  
 Lane, G. R., 117, 165  
 Lang, D. A., 19  
 Langendorff, H., 240, 272, 290, 304, 330, 331, 350  
 Langham, W. H., 75  
 Lartigue, O., 230, 231  
 Latarjet, R., 218, 226, 263, 336  
 Lauder, Y., 39  
 Lawrence, J. H., 29  
 Lawrence, G. H., 277, 304  
 Lawton, E. J., 124, 125  
 Lea, D. E., 3, 9, 24, 50, 52, 53, 54, 55, 57, 58, 60, 61, 62, 64, 65, 66, 67, 68, 69, 75, 82, 83, 85, 93, 95, 130, 162  
 Leblond, C. P., 237, 272  
 Lecloux, J., 72  
 Lecomte, J., 183, 228, 281, 284, 291, 292, 293, 295, 297, 298, 301, 305, 308, 313, 331  
 Lefort, M., 96, 97, 98, 107, 108, 111, 113  
 Lehoul, Y., 177  
 Leinfelder, P. J., 75  
 Lennox, B., 272  
 Leprat, J., 298  
 Levin, A. J., 223  
 Levy, B., 230  
 Lewis, Y. S., 284  
 Liebmann, S., 117, 269  
 Liechti, A., 24  
 Limperos, G., 148, 153, 308  
 Lind, S. C., 32, 35, 37, 38, 39, 80, 81  
 Lipshitz, R., 142  
 Lisco, H., 348  
 Lison, L., 226  
 Little, E. P., 224  
 Livingston, R., 32, 33, 36  
 Logan, M. A., 256  
 Logan, R., 180  
 Loiseleur, J., 235, 301, 303  
 Loos, G. M., 263, 264  
 Looy, G. van, 283  
 Lorand, L., 183  
 Lorenz, E., 316, 330, 336, 340  
 Lorenz, W., 291, 300, 306, 308  
 Lotz, C., 191  
 Louran, M., 230, 231  
 Louran-Pitres, M., 230, 231, 232, 281

## INDEX

- Loutit, J. F., 280, 331, 336, 350  
 Loveless, A., 175, 190, 191, 193, 205  
 Low-Bear, A., 235  
 Ludewig, S., 186, 187, 251  
 Lushbaugh, C. C., 333  
 Lyle, G. G., 253  
 Lyon, M. F., 318
- McCarty, M., 177  
 Macchi, L., 232  
 McCormick, W. G., 234  
 McCulloch, E. F., 234  
 MacDonald, M. R., 141  
 McDonnel, W. R., 41  
 McGilvery, R. W., 175  
 McIndoe, W. M., 180  
 Mack, H. P., 304  
 McKee, R. W., 230  
 Mackey, H., 75  
 McLaren, A. D., 62  
 MacLeod, C. M., 177  
 Magat, M., 39, 103  
 Magee, J. L., 34, 35, 84, 85, 89, 98  
 Magrini, M., 226  
 Mahler, R. F., 345  
 Maisin, J. H., 266, 294, 296, 320, 331, 332, 333, 334  
 Maisin, H., 266, 294, 320, 332, 333, 334  
 Mandart, M., 266, 294, 331, 332, 333  
 Mandel, P., 318  
 Mann, M. M., 82  
 Mann, S., 300, 308  
 Marion, J. P., 42, 127  
 Mar, P. G., 113, 115  
 Marder, S. N., 304  
 Markham R., 143  
 Marks, E. K., 330, 331, 332, 333, 334, 337  
 Marshak, A., 180  
 Marston, R. Q., 340  
 Martin, F. L., 75, 210  
 Mason, G. D., 290, 293  
 Mason, W. B., 231, 239, 243, 274  
 Massart, L., 240  
 Mateyko, G. M., 271, 276  
 Mather, K., 160, 179  
 Matheson, M. S., 109  
 Matney, T. S., 291  
 Matthews, J. J., 301, 304  
 Maxwell, E., 187, 242, 251  
 May, J. P., 271, 272, 281  
 May, M. Le, 242, 256
- Maycock, W. d'A., 280  
 Mayer, S. H., 310  
 Mayneord, W. V., 16, 17  
 Mazzanti, L., 291  
 Mead, J. F., 233  
 Medalia, A. I., 100  
 Meffred, R. B., Jr., 291  
 Meredith W. J., 24, 47, 48, 83, 85, 93, 95, 97, 98, 140  
 Michelson, A. M., 143  
 Mikaelson, K., 229, 253, 292, 310  
 Mikuta, E. T., 241, 246  
 Miller, C. P., 276  
 Miller, J. J., 284  
 Miller, L. L., 253  
 Miller, M., 332, 333  
 Miller, N., 19, 115  
 Millikan, G. A., 214  
 Milne, W. L., 339, 340  
 Minder, W., 24  
 Mira, O. J., 272  
 Mirsky, A. E., 145  
 Mitchell, J. S., 75, 244, 253, 308, 322  
 Mohenny, J. B., 253  
 Moiner, M. M., 270  
 Mole, R. H., 75, 229, 230, 239, 256, 280, 283, 287, 301, 307, 308  
 Momoki, S., 257  
 Montgomery, P. O'B., 348  
 Morehead, F. F., 43  
 Morse, M. L., 293, 303  
 Mori, K., 257  
 Morton, M. E., 229  
 Mosher, W. A., 144, 148, 150, 153, 308  
 Mottram, J. C., 75, 292  
 Moulden, E. J., 215, 303  
 Moulder, P. V., 283  
 Mugard, H., 222, 225, 292, 305, 313  
 Muller, H. J., 66, 67, 350, 351, 352  
 Mund, W., 39  
 Munson, C., 75  
 Muntz, J. A., 240  
 Mylroie, A., 194
- Neary, J. N., 75  
 Needham, D. M., 203  
 Neel, J. W., 352, 353, 354  
 Neujean, G., 283  
 Neukomm, S., 318  
 Newman, R. H., 290, 293  
 Nielsen, G., 331  
 Nims, L. F., 137

## INDEX

- Nira, K., 84  
 Nishina, Y., 8  
 Nizet, A., 248, 249, 295, 301, 305, 328  
 Noe, H. A., 191  
 Noonan, T. R., 245, 254  
 Nybom, N., 66, 75, 310, 311, 315  
 Nye, S. W., 298  
 Ockhler, F., 191  
 Oddie, T. H., 72  
 Ogston, A. G., 195  
 Oliver, R., 256  
 O'Neill, T., 304  
 Ord, M. G., 191, 228, 229, 231, 243,  
     245, 257, 290, 293, 304, 316  
 Pallade, G. E., 185  
 Park, H. D., 223, 224  
 Parr, W., 304  
 Passeau, J., 332, 333  
 Pasteels, J., 179, 226  
 Patersen, E., 243, 301, 304  
 Patt, H. M., 72, 183, 229, 240, 241, 266,  
     271, 275, 280, 290, 291, 293, 294, 295,  
     298, 300, 303, 304, 306, 307, 308, 310,  
     312, 316, 333  
 Patti, F., 220, 290, 291, 303  
 Paull, J., 330, 331, 336, 339  
 Payne, A. H., 180, 253  
 Pearson, 221  
 Peczenik, O., 298  
 Pelc, S. R., 181  
 Pencharz, R., 274, 275  
 Perrin, F., 33  
 Perrin, J. D., 196, 147  
 Perry, S. V., 176  
 Peters, R. A., 183, 184, 203, 232  
 Petersen, D. F., 184  
 Peterson, R. D., 228, 241  
 Petry, E., 72  
 Philips, F. S., 192, 193  
 Phillips, P. H., 251  
 Philpot, J. St. L., 234, 305, 308  
 Phyllis, S. S., 221  
 Pickels, E. G., 136  
 Pirotte, M., 295, 296, 297, 298, 299, 316,  
     319  
 Platt, D., 331  
 Platzman, R. L., 30, 86  
 Pollard, E. C., 47, 60, 62, 144  
 Pollister, A. W., 145, 179  
 Porter, K. R., 176  
 Potter, V. R., 185, 232, 253, 336  
 Pouyet, J., 143  
 Poynter, M., 75  
 Praytor, E. H., 253  
 Prevost-Barnas, A., 39  
 Procopio, J., 276  
 Proctor, B. E., 19, 97, 113, 115  
 Prosser, C. L., 229  
 Puckett, N., 304  
 Pugsley, A. T., 72  
 Pullinger, B. D., 199  
 Quastler, H., 229  
 Rabinowitch, E., 40  
 Rajewsky, B. N., 65, 220  
 Ramioul, H., 270, 298, 300, 321, 346  
 Ramsay, W. J., 37, 79, 80  
 Raper, J. R., 271  
 Rauen, H. M., 145  
 Rayet, P., 293, 295, 297, 301, 308, 316,  
     319  
 Read, J., 66, 71, 75, 117  
 Recd, R., 176  
 Reichmann, M. E., 60, 143  
 Reid, T. R., 336  
 Reim, H., 331  
 Revell, S. H., 163, 198, 199  
 Rhodes, B., 200  
 Richmond, J. E., 247, 248  
 Rigg, T., 102  
 Riley, J. B., 192, 211  
 Risso, O., 81, 83, 107, 115  
 Robinson, J. C., 263, 264  
 Robson, J. M., 190, 330, 331, 332  
 Rodesch, J., 318  
 Roe, E. M. F., 191  
 Rollenaal, H. M., 152  
 Rondoni, P., 235  
 Rose, F. L., 193  
 Rosenblum, C., 39  
 Rosenfeld, F. M., 147, 149, 203  
 Rosenstock, H., 36  
 Rosenthal, R. L., 235  
 Roskam, J., 270, 298  
 Ross, J., 122, 123  
 Ross, M. H., 230  
 Ross, W. C. J., 191, 192, 193, 195, 205  
 Roth, F. J., 272  
 Roth, J. S., 233, 256, 266, 305  
 Rothberg, H., 201  
 Rowbottom, J., 84, 90

## INDEX

- Rugh, R., 220, 221, 224, 230, 294, 348  
 Ruth, H. J., 340  
 Rutherford, Lord, 37  
 Sagedo, E., 276  
 Sallmann, L von, 353  
 Salomon, K., 245, 247, 248, 254  
 Samuel, A. H., 84, 85, 89, 98  
 Saracino, R., 348  
 Sardron, C. H., 143  
 Sargent, S., 191  
 Sarlet, H., 225  
 Sauer, H., 272, 304, 330, 331  
 Sax, K., 165, 224, 304  
 Schechter, D., 175  
 Scherber, F., 316, 317, 318, 319, 334  
 Scheurer, O., 80  
 Schneider, W. C., 185, 253, 336  
 Schneidermann, H., 151, 152, 304  
 Schoenberg, M. D., 151, 152  
 Scholes, G., 75, 149, 151, 152  
 Schubert, G., 116  
 Schwander, H., 144  
 Scott, K. G., 274  
 Scott, O. C. A., 70, 75, 97, 108, 216  
 Segal, G., 237, 272  
 Seki, S. L., 240, 247  
 Selle, W. A., 290, 291, 293  
 Selye, H., 267, 276  
 Setlow, R., 54, 60, 62, 63  
 Shafer, B., 241  
 Shapiro, D. M., 191  
 Shelton, E., 336  
 Sheppard, C. W., 41  
 Sheraga, H. A., 137  
 Sherman, F. G., 254  
 Simmons, E. L., 331, 332, 333, 334  
 Sims, P., 115  
 Sinclair, W. K., 17  
 Singer, T. P., 240  
 Sipe, C. R., 271, 291, 293, 316  
 Slaughter, J. C., 72, 224  
 Smilie, R. M. S., 180  
 Smith, G., 38, 60  
 Smith, D. E., 229, 271, 284, 291, 293, 303, 304, 307, 308  
 Smith, F., 184, 229, 272, 288, 304, 341  
 Smith, H. P., 52  
 Smith, J. D., 143  
 Smith, K. A., 203, 204  
 Smith, K. M., 53, 60  
 Smith, L. E., 36  
 Smith, L. F., 195  
 Smith, T. R., 191  
 Smith, W. W., 272, 288, 304, 340, 341, 342  
 Sobel, E., 253  
 Soddy, F., 37, 79  
 Sonnenblick, B. P., 351  
 Sparrow, A. H., 147, 149, 179, 181, 185, 203  
 Spear, F. F., 156  
 Spehler, H., 237  
 Spiers, F. W., 25  
 Spurr, C. L., 191  
 Stacey, K. A., 144, 195  
 Stannard, J. N., 247  
 Stapleton, G. E., 68, 69, 75, 210, 264, 265, 290, 293, 300, 301, 303, 305, 308, 309, 312  
 Steacie, E. W. R., 39  
 Steadman, L. T., 231, 235, 236, 239  
 Steamer, S. P., 238  
 Steggerda, F. R., 281  
 Stein, G., 43, 89, 91, 102, 115  
 Steinhardt, J., 144  
 Stern, K. G., 145  
 Stevens, C. M., 194  
 Stich, H., 180  
 Stock, C. C., 193  
 Stocken, L. A., 228, 231, 243, 245, 257, 290, 293, 304, 316  
 Stone, W. S., 292  
 Storer, J. B., 303, 333  
 Straube, R. L., 266, 271, 293, 294, 295, 303, 304, 306, 307, 308, 310, 333  
 Sullivan, R. L., 223  
 Supplee, H., 239, 241  
 Sussman, A. S., 186, 251, 252  
 Sutton, H. C., 90, 96, 107, 108, 111, 113, 140, 150  
 Svedberg, T., 63, 128, 135, 136  
 Swift, H., 179  
 Swift, H. H., 226  
 Swift, M. N., 183, 275, 291, 304, 340  
 Sworaki, T. J., 91  
 Syverston, J. T., 272  
 Szafarz, D., 180  
 Taggart, J. V., 175  
 Tansley, C., 354  
 Tahmusian, T. N., 75, 241, 257  
 Tate, J. T., 82  
 Taylor, B., 146, 148, 149, 150, 151, 203

## INDEX

- Taylor, H. F. W., 195  
Taylor, H. S., 30, 36, 38  
Taylor, J. H., 180  
Teich, S., 269  
Teller, E., 33  
Tew, J. T., 215, 303  
Thiersch, J. B., 193  
Timmis, G. M., 193, 202  
Thoday, J. M., 69, 71, 75  
Thomas, S. F., 274, 275  
Thompson, E. C., 272, 342  
Thompson, H. E., 231, 235, 236  
Thomson, J. J., 37  
Thomson, J. F., 237, 241, 242, 243, 246  
Timoféeff-Ressovsky, N. W., 66, 75  
Tobias, C. A., 29, 59, 69, 73, 75, 220,  
    226  
Todd, A. R., 143  
Tourneur, R., 298  
Tourtelotte, W. W., 237, 242, 243  
Treadwell, A. de G., 304  
Tribondeau, L., 156  
Trowell, O. A., 179, 214, 273, 281, 309  
Truhaut, R., 298  
Tschaperoff, I. C. C., 113, 115  
Tuchmann-Duplessis, H., 276  
Tyree, E. B., 183, 229, 271, 275, 291,  
    293, 303, 304, 307, 308  
Tytell, A. A., 256  
  
Uphoff, D., 336, 340  
Uri, N., 104  
  
Valencia, T., 67  
Valkenburg, P. A. van, 253  
Velley, G., 235, 301, 303  
Vendrely, C., 179  
Vendrely, R., 177, 179  
Verly, W., 295, 296, 298  
Vidovic, V., 216  
  
Vigne, J., 234  
Vintemberger, P., 184, 246  
Vivario, R., 72  
Vliers, M., 298  
Voegelin, C., 186  
Vogel, H. H., 75, 310  
Volkin, E., 239  
  
Wahrhaftig, A. L., 36  
Wakelin, R. W., 232  
Wallenstein, M., 36  
Walpole, A. L., 193  
Wang, S. C., 294  
Waters, W. A., 100  
Watson, J. D., 143, 144  
Watson, R., 231  
Weber, R. P., 281  
Weiss, J., 81, 89, 91, 102, 115, 149, 151,  
    152  
Weiss, R. J., 270  
Wellers, G., 191  
Werder, A. A., 272  
Werker, B. C., 274, 275  
Wertz, E., 72  
West, E. S., 228, 241  
Weymouth, P. P., 336, 339  
Whipple, G. H., 231, 239, 243  
Whitcher, S. L., 114  
Wilkinson, J., 19  
Williams, E. K., 349  
Wolff, E., 312  
Woods, H. J., 134  
Wyss, O., 292  
  
Yagoda, H., 23  
  
Zahl, P. A., 243  
Zamenhof, S., 143  
Zimmer, K. G., 66, 75  
Zirkle, R. E., 73, 75, 171, 330, 331, 332

## SUBJECT INDEX

Italicized page numbers indicate that the subject is referred to on subsequent pages

- Absorption coefficient, atomic, 6  
contribution of Compton effect, 12  
pair formation, 12  
photoelectric effect, 7, 12  
definition of, 6  
electronic, 6  
and energy of  $\alpha$ - or  $\gamma$ -radiation, 12  
mass, see Mass absorption coefficient  
magnitude of and chemical con-  
stitution, 7  
Accidental massive irradiation, 348  
Acentric fragment, 166 fig. 15  
*Acetabularia mediterranea*, 180  
Acetaldehyde, 112  
Acetic acid, 112, 301  
Acetate, changes in metabolism of, 245  
increase in metabolism of in proto-  
zoon, 233  
incorporation in haemoglobin, 248  
Acetone, 112  
Acetylcholine, 186, 256  
Acetylene, polymerization of, 38  
Acids, decarboxylation of, 41  
reaction with radiomimetic chemicals,  
195  
Acrylonitrile, 100  
polymerization of, 96  
ACTH, 267, 272, 281  
determination of, 269  
production of radioresistance by, 271  
Action at a distance, 162  
Activation of enzymes, 245  
Adaptation syndrome, 269  
Addison's disease, 269  
Adenocarcinoma, mammary, 318  
Adenosinetriphosphatase, see ATPase  
Adenosinetriphosphoric acid, see ATP  
Adrenal, 267  
changes in ascorbic acid content, 274  
cholesterol content, 274  
detection of changes in, 269  
effect of cysteamine on, 298  
hyperactivity, 274  
influence on enzymes, 246  
of shielding, 272  
role in radiation sickness, 267  
Adrenal cortex, 268  
changes in, 276  
protection of, 318  
Adrenalectomy, 237, 246, 271, 273, 276,  
306  
Adrenaline, 215, 272, 303, 311  
Adrenocorticotrophic hormone, see  
ACTH  
After-effect, in irradiation of trypsin,  
142  
in DNA degradation, 148  
influence of oxygen, 148  
glutathione, 149  
Anaemia, production of by radiation,  
285  
Air, mass absorption coefficient of, 11  
Alarm reaction, 272  
Alcohol, 51  
effect of irradiation on, 41  
protection by, 301  
Algae, 180, 224  
Alkylating agents, see Radiomimetic  
chemicals  
*Allium cepa*, 161, 198  
protection of, 298, 310  
Alloxazine adenine dinucleotide, 141  
Allylamine, 302  
Allylthiourea, 128  
 $\alpha$ -rays, 13, 89  
chemical reaction of, 81  
dissociation of haemocyanin by, 135  
comparison of effectiveness with x-  
rays, 98  
distribution of ions around, 93  
effect of on water, 81  
formation of hydrogen peroxide by,  
74, 80  
ionization by, 20

## SUBJECT INDEX

- $\alpha$ -rays—(Cont.)  
 irradiation, calculation of target size from, 57  
 lethal dose for mice, 220  
 limiting concentration, 97  
 production of chromosome aberrations by, 71, 171  
 range of, 3  
 source of, 3
- Amines, inhibition of action of mustard gas by, 190  
 protection of mammals by, 302  
 reaction with radiomimetic chemicals, 195
- Amino-acid oxidase, 139
- Amino-acids, deamination of by x-rays, 113  
 presence in invertebrate and radiosensitivity, 225  
 presence in tumours and radiosensitivity, 225  
 protection of mammals by, 302
- Aminobenzoic acid, 302
- Aminopropiophenone, 303
- Aminostilbene, 191
- Ammonium chloride, 302
- Amoeba, 191, 220
- Amoeba proteus, 342
- Amphibians, radiosensitivity of, 221
- Anaphase, 158
- Anaphase bridges, see Chromosome bridges
- Anemone sulcata, 233
- Aniline, 128, 302
- Annelida, 221
- Anophthalmia, 312
- Anoxia, chronic and radiosensitivity, 342  
 influence on chemical protection, 215, 310  
 influence on radiosensitivity, 209  
 relation to chemical protection, 308
- Anorexia, 287, 345
- Antibiotics, effect of in radiation sickness, 285
- Antibodies, changes in, 284  
 decreased formation of, 192, 238
- Antifolic acids, interference of DNA synthesis by, 256
- Antigens, 60
- Antihistamines, 281
- Antivitamin, 191, 256
- Aqueous solutions, aerated reactions in, 104  
 without oxygen, reaction in, 99
- Aqueous systems, radiation chemistry of, 77
- Arbacia punctulata*, 240, 247, 263
- Arginine, 302
- Arsenical substances, 307
- Arsenite, oxidation of, 92
- Arthropoda, 221
- Ascites tumours, 70, 75, 181, 255  
 variation in resistance with oxygen tension, 217
- Ascopal, see Action at a distance
- Ascorbic acid, 54, 72, 102, 109, 115  
 in adrenals, 270, 274  
 contents of different plants, 235  
 depletion of by cysteamine, 298  
 in liver, 270  
 protection by, 303  
 protective action of, 235  
 relation to radio resistance, 235  
 in suprarenals, 275
- Aspartic acid, 302
- Aspergillus terreus*, 75
- Associated volume method, 57, 61
- Asthenia, 345
- ATP, 175, 342  
 changes in ascites tumours, 255  
 synthesis, changed by irradiation, 254
- ATPase, 140, 187, 242, 243
- Atomic energy, dangers from, 350  
 explosions, 346  
 results of, 352
- Atropine, 288
- Attagenus*, 223
- Auger effect, 7, 30
- Aureomycin, 285
- Autoradiograph, 181
- Azide, protection by, 292
- Bacillus erythropolis*, 186  
*megatherium*, 263  
*mesentericus*, 220
- Bacteria, influence of medium on radiosensitivity, 68  
 irradiation when frozen, 66  
 killing of application of target theory, 67  
 oxygen effect, 68  
 protection of, 68  
 recovery of after irradiation, 69

## SUBJECT INDEX

- shape of survival curve, 68  
 Bacteriophage, 234  
     influence of oxygen on radiosensitivity, 218  
 BAL, 309  
 Barley, 66, 75  
     influence of oxygen on radiosensitivity, 66  
 Bectaptan, see Cysteamine  
 Benzene, 42, 51, 89, 91, 109  
 Benzoic acid, 175  
 Bergonié-Tribondeau's Law, 214  
 Beri-beri, 183  
 $\beta$ -rays, 2  
     artificial radioactivity from, 5  
     from radio-active isotopes, 4  
     range of, 3  
 Betatron electrons, chemical and biological reactions produced by, 115  
 Binding energy, 7  
 Biochemical lesion, 184  
 Biochemistry of mitosis, 175  
 Biochemistry, pathological, 228  
 Birds, irradiation of embryo, 312  
     radiosensitivity of, 222  
 Bisulphite, 303  
 Bisulphite-binding substances, 232  
 Blood, changes in, 350  
     coagubility, 284  
     after irradiation, 286  
     synthesis, 249  
     clotting of, 283  
     coagulation defect in, 191  
     sugar content of, 231  
     urea content of, 238  
 Blood corpuscles, isolated nuclei from, 171  
*Boletus edulis*, 186  
*Bombyx mori*, 184, 264  
 Bone-marrow, changes of haemoglobin  
     synthesis in, 247  
     *in vivo* activity, 247  
     protection of, 310  
     shielding of, 332  
     stimulation of by irradiation, 248  
 Bone-marrow grafts, 333  
     homogenates, regeneration by, 336  
         effect on lymphoma, 339  
 Broad bean, see *Vicia faba*  
 Brain, 245  
     incorporation of glycine by, 253  
     influence of radiation on, 348  
 Bronchial carcinoma, 322  
 Butyl hydroperoxide, 200  
 Butyric acid, 112  
 Cage effect, 40, 122  
 Calcium, changes in concentration of, 239  
 Cancer, 198, 202, 253, 288, 304, 318, 322, 339, 353  
     radiotherapy of, 35, 156, 216, 321, 329  
 See also Carcinogenic action, Tumours  
 Cancer patients, radiation sickness in, 344  
 Capillaries hepatic, 254  
 Capillary, permeability increase of, 281  
 Caprylic acid, 301  
 Carbohydrate metabolism, 176, 183  
     after irradiation, 230  
 Carbon atoms, ionized, 5  
 Carbon monoxide, 303  
     sensitization by, 304  
 Carboxylic acids, protection by, 301  
 Carboxypeptidase, 98, 139  
     effect on *in vivo*, 247  
     inhibitor destruction of, 247  
 Carcinogenic action, 350  
     effect of cell homogenates, 339  
         lead shielding, 330  
     of radiomimetic chemicals, 191  
 Carcinogenic hydrocarbons, 191  
 Castration, 229  
 Catalase, 62, 63, 139, 140, 186  
     activity in liver, 257  
     in algae influence of radiation, 224  
     changes *in vivo*, 253  
     influence of irradiated serum on, 257  
     inhibitors, protection by, 292  
 Catalytic effect of inert gases, 39  
 Cataract, 288, 349, 353  
 Cathepsins, see Carboxypeptidase  
 Cathode rays, see  $\beta$ -rays, 46  
 Cavity, ionization chamber, 17  
 Cell constituents, replacement of, 175  
 Cells, fractionation of by centrifugation, 337  
 Cellular barriers, breakdown of, 76  
 Centromere, 158, 167  
 Cerenkov radiation, 11  
 Ceric salts, 101, 102  
     reduction of, 84, 89  
 Chain termination, 116

## SUBJECT INDEX

- Charge neutralization, 35  
 Charge transfer, 35  
     and cluster formation, 38  
     in gases, 38  
     in liquids, 42  
 Chelating agents, protection by, 300  
 Chemical protectors, see Protection, chemical  
 Chiasmata, 160 fig. 4  
 Chicks, 238  
 Chloramphenicol, 285  
 Cholesterol, 235, 274  
     in adrenals, 270  
 Choline, 266, 302  
 Choline oxydase, 251  
 Cholinesterase, 186, 256, 280  
     'pseudo', 256  
 Chromatids, 158  
 Chromatid breaks, 156, 160 fig. 16, 162  
     definition of, 163  
 Chromatid exchange, 166 fig. 16  
     isochromatid exchange, 166 fig. 16  
 Chromatin, 145  
 Chromocentre, 158, 160 fig. 3  
 Chromosome(s), changes in sensitivity of, 166  
     constitution of, 176  
     dicentric, 167  
     duplication of, 159  
     exchange, 166 fig. 16  
     nucleo-proteins of, 146  
     period of maximum sensitivity, 173  
     permanent effects, 162  
     from salivary gland, 160 fig. 3  
     spiral structure of, 160 fig. 2  
     spiralization of, 178  
     temporary effects, 162  
     variation in DNA content, 178  
 Chromosome abnormalities, 136  
     application of target theory to, 69  
     influence of oxygen on radiosensitivity, 210  
     oxygen effect on, 71  
     produced by  $\alpha$ -particles, 71  
         by neutrons, 71  
         by oxygen only, 212  
     protection by cyanide, 292  
         by cysteine, 310  
     by radiomimetic chemicals, 191, 198  
 RBE of, 75  
     in tumour cells, 162 fig. 9  
*Vicia faba*, 160 fig. 10, 163  
 Chromosome breaks, 156, 162, 171  
     biochemical interpretation, 175  
     classification of, 163  
     influence of dose rate on, 165  
     influence of interrupting dose, 165  
     localization of by different treatments, 198  
     mechanism of, 171  
     and mutagenic action, 169  
     by oxygen, 169  
     RBE of different radiation, 173  
     restitution of, 164, 167  
     reunions of, 166  
     spontaneous, 169  
     subsequent fate of, 167  
 Chromosome bridges, 166 fig. 15, 167  
 Chromosome effects, physiological, 160 fig. 6, 162  
 Chromosome interchanges, 164  
 Chromosome loop, 168  
 Chymotrypsin, 313  
 Cicatrization, 263  
 Ciliates, 292  
 Cirrhosis, 238  
 Citric acid, accumulation of after fluoroacetate, 232  
     changes in synthesis in, 232  
     destruction of in liver, 233  
*Cladophoropsis*, 224  
 Clotting, see Blood clotting  
 Cluster formation, 37  
 Clusters, 93  
 Coagulation of colloids, 116  
 Coal gas, 303  
 Co-enzyme A, 175  
     inhibition of *in vivo*, 241  
     relation to protection by cysteamine, 295  
         structure of, 242  
 Co-enzyme I, 342  
 Cobalt-60,  $\gamma$ -rays from, 2  
 Co-carboxylase, 182  
 Coelenterates, 223  
 Colchicine, 169  
 Cold, influence on radiosensitivity of mammals, 216  
 Colloids, effect of radiation on, 116  
*Colpidium*, 220  
 Competition factor, 195, 196  
 Competition for free radicals, protection by, 312

## SUBJECT INDEX

- Compton effect, 7  
 influence of wavelength of x- and  $\gamma$ -rays on, 8  
 magnitude of, and chemical constitution, 8
- Compton electrons, energy distribution by, 9
- Corticoids, 267
- Coricosteroids, synthesis of, 270
- Corticotropic pituitary hormone, 276
- Cortisone, 191, 237, 271, 304  
 influence on radiation sickness, 272  
 methods of measurement, 270  
 production of radioresistance by, 271
- Cosmos sulphurens*, 235
- Crossing-over, 160
- Crosslinking hypothesis, 204
- Crosslinking, inter- and intra-molecular, 204  
 interference by energy transfer, 129  
 mechanism of, 126  
 of polymers, 121  
 of polymers in aqueous solution, 130
- Crosslinks, formation of, 40  
 disulphide, 135
- Cross-resistance, 269
- Crotonic acid, 109
- Ctenophora*, 221
- Curie unit, 15
- Cyanide, combined effect of with glutathione, 292  
 mutagenic action of, 169  
 protection by, before and after irradiation, 305  
 of mammals by, 291  
 protection of bacteria by, 309  
 resistance to and radiosensitivity, 313  
 of Infusoria to, 222, 225  
 sensitizing effect of, 292
- Cyclohexane, 42
- Cyclopia*, 312
- Cyclotrons, 349, 353
- Cystamine, administration of, 295  
 liberation of histamine by, 297, 312  
 metabolism of, 296  
 protection of mammals by, 293
- Cysteamine, appearance in urine, 299  
 combined effect of with cystine, 295  
 cytotoxic action of, 298  
 derivatives of, 294  
 distribution of in different tissues, 297
- effect on leucocyte count, 317  
 testis, 318
- influence on distribution of ascorbic acid, 298  
 liver glycogen, 230  
 oxygen concentration in tissues, 310  
 weight loss, 287, 316
- metabolism of 296
- protection against cataract, 353  
 of DNA by, 148  
*E. coli*, 305  
 enzymes by, 240  
 of liver, 320  
 of mammals by, 293  
 against nitrogen mustards, 298  
 against oxygen poisoning, 212  
 of polymers against direct action, 128  
 crosslinking, 131  
 against sterilization, 319  
 of suprarenals, 275  
 tumours, 318
- restoration by, 266
- treatment for radiation sickness, 342
- Cysteine, 102, 109, 137, 309  
 combined effect of with cysteamine, 295  
 decarboxylation of, 296  
 effect of adrenalectomy on protection, 306  
 influence on oxygen concentration in tissues, 310  
 of pH on decomposition of, 114  
 irradiation of, 114  
 protection by, 293  
 against cataract, 353  
 of linoleic acid, 233  
 against nitrogen mustards, 298
- Cysteine chromosome abnormalities, protection against, 310
- Cystine, 297, 302
- Cystine, protection of enzymes by, 240
- Cytochrome oxydase, 242, 252
- Cytoplasm, 157  
 action of chemicals on, 191  
 breakage by protons, 171  
 changes during mitosis, 159  
 RNA synthesis in, 180
- Cytoplasm-nucleus, relative sensitivity of, 250
- Cytotoxic effects, definition of, 202

## SUBJECT INDEX

- Deactivation of excited molecules, 40  
 D-amino-acid-oxidase, 139  
 Death by extreme irradiation, 280  
 Degenerative changes, 191  
 Degradation of polymers, 121  
     in aqueous solution, 131  
     mechanism of, 126  
 Dehydroascorbic acid, 235, 270  
 Dehydrogenase activity, 242  
     phosphoglyceraldehyde, 140  
     yeast alcohol, 139  
 Delay of radiation effects, 183  
 $\delta$ -rays, 23, 93, 98  
 Density of ionization, see Specific ionization  
 Deoxycorticosterone acetate, see DOCA  
 Deoxyribonuclease, 60, 186, 338  
 Deoxyribonucleic acids, see DNA  
 Depth, influence of dose on, 28  
     at which dose is maximal, 28  
 Deuterons, 5  
 Diarrhoea, 345  
 Dibenamine, 288  
 Diethylalanine, 302  
 Diethylthiocarbamate, 300  
 Diffusion of radicals from  $\alpha$ -and electron-tracks, 95  
 Digestive disturbances, 345  
 Digestive tract, 288  
 Dihydroxyphenylalanine, 302  
 Diketogluconic acid, 235, 270  
 Dilution effect, 47  
 Dimercaptopropanol, 300  
 Dimethylamine, 302  
 Diphosphopyridine nucleotide, 342  
 Diploid cells, inactivation of, 59  
 Direct action, application to chromosome breaks, 171  
     comparison with indirect, 51  
     definition of, 46  
     experimental detection of, 47  
     influence of  $\text{HO}_2$  radicals, 104  
     *in vivo*, 64  
     protection against, 127  
     relationship of dose in, 49  
     on synthetic macromolecules, 121  
 Distant effects, 328  
 Disulphide bonds, 114, 135  
 Dithionite, 303  
 Dithiophosphate, 308  
 Ditopic, 73  
 DNA, 60, 142  
     change of shape by nitrogen mustards, 203  
     chemical changes produced on irradiation, 151  
     as chromosome constituent, 176  
     combination with protamine, 145  
     content, influence on radiosensitivity, 181  
     degradation of by x-rays, 147  
     dissociation of by urea, 144  
     effect of irradiation *in vivo*, 153  
     esterification of phosphate groups, 205  
     influence of oxygen on inactivation of, 218  
     molecular weight of, 144  
     protection against degradation, 148  
     protection of *in vivo*, 153  
     reaction with radiomimetic chemicals, 203  
     reduction of protein combining capacity, 205  
     relation to genes, 177  
         protein synthesis, 177  
     sensitivity to hydrogen peroxide, 149  
     synthesis, 173  
         in actics tumours, 255  
         inhibition of by x-rays, 181  
         interference by radiomimetic chemicals, 203  
     transforming factor, 177  
     treatment for radiation sickness, 348  
     twin spiral structure of, 143  
     viscosity of, 143, 150  
 DOCA, 237, 268, 273  
     production of radioresistance by, 271  
     protective action of, 272  
 Dog, 220  
     haemoglobin synthesis in, 249  
 Dominant lethals, 201  
 Dose, maximum permissible, 349, 352  
     variation with depth for electrons, 29  
         heavy particles, 29  
         x-rays, 28  
 Dose rate, influence on chemical reactions, 115  
     chromosome breaks, 162, 165  
     mitosis, 163  
 Dose 37 per cent, definition of, 50  
     effect of concentration on, 52  
     for direct and indirect action, 52

## SUBJECT INDEX

- Dosimeter, calorimetric, 19  
 ferrous sulphate, 19
- Dosimetry, chemical, 18  
 physical, 17
- Double bond, substitution at by OH radicals, 100
- Drosophila*, 170, 180, 200, 201, 318  
 eggs, killing of, 75  
 induced mutations, 156  
     influence of oxygen on, 67, 213  
 sex-linked lethal mutations, 116
- Effect at a distance, 328
- Eggs, delay of radiation effects in, 184  
     restoration of by cooling, 264
- Electric double layer, 117
- Electrokinetic potential, 117
- Electrolyte concentration, changes in, 239
- Electron thermal, 22  
     tracks, 94  
     volt, definition of, 2
- Electron(s) from photoelectric effect, 7  
     produced by Compton effect, 9  
     specific ionization of, 21
- Electron-positron production, 10
- Electrophilic, 194
- Electrophoretic velocity, 117
- Embryo, fish, 160 fig. 5  
     protection of, 312
- Embryonic development, 246  
     nerve cells, 348
- Energy to form an ion pair, in air, 15, 18  
     and ionization potential, 30  
     in liquids, 39  
     required to crosslink polymers, 122  
         to degrade polymers, 122, 126
- Energy loss by  $\alpha$ -particles, 13  
     electrons ( $\beta$ -rays), 13  
     ionizing radiations, 6  
     protons, 13
- Energy, metabolic interruption of, 182
- Energy transfer, 33, 42, 46, 127  
     and direct action, 47  
     in liquids, 42  
     within macromolecules, 128  
     protection of polymers, by, 127  
     in protein, 128  
     in solids, 127  
     and target size, 130  
     theory, 62
- Enzymatic activity, increase in, 187  
     after irradiation, 245
- Enzymes, activation of *in vivo*, 245  
     blocking of and protection, 313  
     inactivation of, 139  
         by free radicals, 139  
     inhibition of *in vivo*, 256  
     molecular weight of, 60  
     release from mitochondria, 186  
     sulphydryl, inactivation of, 240
- Epoxides, 192  
     chromosome breaks by, 199
- Ergothioneine, 294, 307
- Erythema, 329
- Erythrocytes, changes in nucleoprotein of, 153  
     in oxygen consumption, 250
- Escherichia coli*, 75  
     influence of oxygen on radiosensitivity, 210  
     mechanism of protection, 309  
     mutagenic changes in, 212  
     protection by cyanide, 291  
         cysteamine, 305  
         mercaptoethanol, 305  
     restoration of, 264
- Ethanol, 309
- Ethanolamine, 302
- Ethylamine, 302
- Ethylene diamine, 302  
     imines, 192  
         clinical application of, 202
- Ethoxycaffeine, 199
- Euchromatic regions, 178
- Excitation, by  $\alpha$ -particles, 30  
     contribution to degradation, 127  
     dissociation of molecules by, 32  
     in liquids, 40  
         deactivation of, 40  
     of molecules, 31  
     produced by ionizing radiations, 30  
     and target theory, 56  
     of water, 83
- F-centres, 43
- Fat, effect of radiations on 41, 233, 237  
     liver, 237
- Fat content, changes in animal, 236  
     metabolism, changes in, 235  
     peroxides, 233
- Fatty acids, influence on hydrogen peroxide formation, 110

## SUBJECT INDEX

- Fatty acids—(Contd.)  
 protection of polymers by, 110  
 Feeding, forced, 288  
 Fenton's reagent, 100  
 Ferric sulphate, 169  
 Ferrocyanide, oxidation of, 92  
 Ferrous sulphate dosimeter, 19  
 oxidation of, 89, 105  
     influence of oxygen concentration,  
         106  
     post irradiation oxidation, 90  
 Fertility, stimulation of, 351  
 Feulgen staining, 179  
 Fibrinogen, 135, 137  
 Fibrinolytic activity, 284  
 Fluoride, 307  
 Fluoroacetate, 307  
 Fluoroacetic acid, 232  
 Folic acid, 286, 304  
     antagonists, 191  
 Formaldehyde, 112, 201  
     decomposition of, 92  
 Formate, 292, 309  
 Formic acid, 102, 109, 112, 301  
     decomposition of, 92, 111  
 Forward reaction, 86, 98  
     influence of specific ionization, 89  
     yield of, 90  
 Fractionation of cells by centrifugation, 337  
 Franck-Condon principle, 32  
 Free radicals, 31, 34  
     formation of, 81  
     formed in water, 81  
     ions, 34  
     mechanism of formation in water,  
         82, 85, 103  
     protection against by competition,  
     role of *in vivo*, 73 [312]  
 Freezing test and indirect action, 49  
*Fritillaria pallidiflora*, 160 fig. 4, 111  
 Frogs, 137, 183, 220, 251  
 Frozen material, irradiation of, 65  
 Fructose, 301  
 Fumaric acid, 109
- G value, definition of, 36  
     in ferrous sulphate dosimeter, 19  
     see Yield
- $\gamma$ -rays, 1  
     artificial radio activity from, 5  
     wavelength and voltage, 2
- $\gamma$ -ray irradiation, calculation of target size from, 57  
 Gametes, 160  
 Gases, evolution from polymers, 122  
     reactions in, 37  
     See also Inert gases  
 Gel point, 123  
 Gels, reactions in, 43  
 Gelatine, 53  
 Gene codes, 177  
     mutation, see mutation size, 67  
 Genes, 163  
 Genetic changes, 163  
     influence of atomic explosion on, 353  
     death, 351  
*Gladiolus*, 235  
 Globin, synthesis of, 247  
 Glucosamine, 302  
 Glucose, 148, 301  
     absorption of, 231  
     changes in metabolism of, 245  
     plasma, 231  
     protection by, 54  
 Glutamic acid, 302  
 Glutathione, 102, 115, 140, 149, 316, 318  
     changes in blood cells, 228  
     combined effect with cyanide, 292  
     oxidation of *in vivo*, 241  
     protection by, 293  
 Glycaemia, 232  
 Glycerophosphate, 185  
 Glycine, 175, 302  
     changes in metabolism of, 245  
     deamination of, 98  
         in aqueous solution, 112  
     incorporation in ascites tumours, 255  
         haemoglobin, 249  
 Glycine ethyl ester, 302  
 Glycogen, changes in liver, concentration of, 230  
     influence on synthesis of, 231  
     liver changes by cysteamine, 299  
 Goat, 220  
 Goldfish, 220  
 Gonadotrophic hormone, 267  
 Graaff, Van de, generator, 2, 3  
 Gramme roentgen, 16  
 Granulocytes, 202, 286  
     changes produced by irradiation, 282  
 Grasshopper nymph ovarioles, 75  
 Grid irradiation, 329

## SUBJECT INDEX

- Growth, cessation of, 238  
 decrease in, 316  
 hormone, see STH  
 influence of atomic explosion on, 353  
 inhibition by radiomimetic chemicals, 191
- Guinea-pig*, 220, 229, 257, 276  
*Gynandromorphs*, 201
- Habrobracon*, 170, 223  
 Haematopoietic organs, regeneration of, 316, 334  
 shielding of, 333
- Haematopoietic regeneration factor, 336
- Haemocyanin, 60, 128, 135, 136  
 Haemoglobin, degradation of, 116  
 synthesis of, 247  
 inhibition of by cysteamine, 299  
 and regeneration factor, 341
- Haemorrhages, in radiation sickness, 283  
 petechial, 284
- Hair, greying of, 191  
*Halimeda*, 224  
 Hamster, 220  
 Haploid cells, inactivation of, 59  
*Helix pomatia*, 135  
 Heparin, 284  
 Heterochromatic region, 178, 199, 200  
 Heterochromatin, 179, 199  
 Hibernation, delay of radiation effects, by, 184  
 High energy bursts, biological effects of, 115  
 chemical reactions of, 115
- Hippuric acid, 175  
 Histamine, 302, 307  
 liberation of, 281  
 by cystamine, 297  
 of and protection by, 312
- Histidine, 113, 302  
 Histones, 145, 152  
 synthesis of, 238
- Histological changes, effect of protectors on, 319
- HN2, 198, see also Nitrogen mustards
- $\text{HO}_2$  radicals and chemical protection, 298, 314  
 competition for, 315  
 degradation of polymers by, 133  
 donation of oxygen by, 105
- reaction of, 104  
 with organic compounds, 105
- Hodgkin's disease, 202
- Homocysteine, 293
- Homogenates of cells, preparation of, 336  
 regeneration by, 336
- Hot spots, 2
- Humans, lethal dose, 346  
 observations on, 344
- Hyaluronic acid, 151  
*Hydra*, 223
- Hydration, influence on radio resistance, 72
- Hydrogen, combination with oxygen by radon, 80  
 from decomposition of water, 79
- Hydrogen atoms, 82  
 abstraction of by OH radicals, 100  
 distribution of in tracks of  $\alpha$ -particles, 93  
 x-ray tracks, 93  
 evidence against formation of in water, 102  
 formation of in gases, 38  
 inactivation of catalase by, 140  
 reactions with protons, 102  
 reactivity of, 101
- Hydrogen peroxide, 169, 182  
 and biological action of  $\alpha$ -particles, 74  
 decomposition of, 84, 89  
 effect on DNA, 149  
 energy to dissociate, 84  
 formation of, 107  
 in aerated water, 89, 107  
 influence of added substances, 109  
 dose, 108  
 specific ionization, 108  
 by  $\alpha$ -particles, 80, 111  
 inactivation of phage by, 149  
 stability of, 234
- Hydrogen sulphide, 114
- Hydroperoxides, see Peroxides
- Hydroquinone, 109
- diphosphate, 322  
 oxydase, 257
- Hydrosulphide, 293
- Hydrosulphite, 303
- Hydroxyl ion, 102
- Hydroxyl radicals, see OH-radicals
- Hydroxylamine, 292

## SUBJECT INDEX

- Hydroxyquinoline, 128  
Hydroxytryptamine, 225, 303, 311  
Hydroxytyramine, 302  
*Hyoscyamus niger*, 235  
Hyperglycaemia, 231, 235, 239, 268  
Hypocalcaemia, 239  
Hypophysectomy, 246, 273  
Hypothalamic centres, 306  
Hypothyroidism, 308  
Hypothyroidy, 229
- Inactivation, comparison of direct and indirect, 51  
exponential relationship to dose, 50  
influence on dose, 49
- Indirect Action, comparison with direct, 51  
definition of, 46  
experimental detection of, 47  
influence of RBE on, 74  
*in vivo*, 72  
*in vivo*, poison theory, 72  
quantitative theories of, 73  
low efficiency for virus inactivation, 64  
and multiple hit effects, 74  
oxygen effect, 209  
relationship of dose on, 49  
on synthetic macromolecules, 130
- Impurities, protection of viruses by, 52
- Inert gases, catalytic effect of in gas reactions, 39  
charge transfer by, 39
- Infection, decrease by shielding, 334  
gastro-intestinal, 285  
result of irradiation, 284  
leucopenia, 283
- Infra-red, sensitization of chromosomes by, 201
- Infusible, rendering polymers, 123
- Infusoria, 223, 305  
relation between resistance to cyanide and x-rays, 222, 225
- Inhibition of enzyme *in vivo*, 256
- Insecticidal action of ionizing radiation, 223
- Insects, radiosensitivity of, 222
- Insulin, 60  
influence on lipaemia, 236
- Intensification of radiation effect, 322
- Interchanges, see Chromosome interchanges
- Interkinesis, 158  
Internal conversion, 33, 40, 128
- Interphase, 158
- Interrupted irradiation, influence on chromosome breaks, 165
- Intestinal mucosa, 319
- Intestine, pyknosis nuclei of, 319  
radiation damage of, 231  
shielding of, 332
- Intracellular structures, breakdown of, 187
- Inversion, 167, 201
- Invertebrates, radiosensitivity of, 221
- Iodate, 102
- Iodine, 81  
atoms, 314  
uptake of thyroid, 253
- Ion cluster, detection of in cloud chamber, 22, 61
- Ion pairs, detection of in cloud chamber, 22
- Ions, dissociation of, 35  
distribution of in space, 22  
formation, 34  
reaction of, 34  
with neutral molecules, 36  
spatial distribution of in water, 91
- Ionic yield, definition of, 36  
in gases, 37  
influence of excitation on, 84  
in water, 81  
see Yield
- Ionization chamber, 17  
density, see specific ionization  
in liquids, 23  
multiple, 30  
potential, 30
- Isotopes, 4  
measurement of radioactivity of, 15  
use as tracers, 181, 244, 247, 253
- Jensen's rat sarcoma, 257
- K factor, 15
- Ketoglutaric acid, 243, 301
- Ketosteroids, estimation of, 269  
excretion of, 277
- Kidney, shielding of, 332  
homogenates, oxygen consumption by, 251
- Kinetics of radiochemical reactions, 96
- Krebs cycle, 309

## SUBJECT INDEX

- Lamellibranch (*Spisula solidissima*), 224  
*Lasioderma*, 223  
 Lead shielding, see Shielding  
 Lecithin, changes in metabolism of, 244  
 Lens, epithelium of, 353  
 Lethal dose for humans, 346  
     for various animals, 221  
 Leucocytes, changes in and infection, 283  
     as control, 349, 352  
     count, effect of cysteamine on, 317  
 Leucopenia, 229, 281, 283, 310, 346, 348  
     effect of cysteamine on, 316  
 Leukaemia, 202, 353  
     influence of atomic explosion on, 353  
     myeloid, 202  
 Lily seeds (*Lilium regale*), 224  
 Limiting concentration, 97  
     dependence on specific ionization, 97  
     of oxygen dissolved in water, 97  
     and radical recombination, 92  
*Limulus polyphemus*, 136  
 Linear accelerators, 2, 353  
 Linoleic acid, oxidation of, 233  
 Lipaemia, 235  
 Lipids, see Fat  
 Liquids, G value for radical formation in, 39  
     ionization density in, 23  
     reactions in, 39  
 Liver, catalase activity in, 257  
     changes in ascorbic acid content by cysteamine, 298  
     enzymatic activity, 246  
     glycogen content, 230  
     metabolism of, 230  
     citric acid content of, 232  
     effect of cysteamine on, 299  
     fats, 237  
     histological changes in, 320  
     homogenates, oxygen consumption by, 251  
     implantation of, 333  
     incorporation of phosphorus in, 254  
     oxidation of cysteamine by, 296  
     proteins, 245  
     radioresistance and polyploidy, 226  
     radiosensitivity of, 255  
     shielding, 332  
 Localization of energy, 127  
*Loligo pealii*, 223  
 Los Alamos, 347  
 Lundsgaard contracture, 182, 185  
 Lymph glands, cervical, 328  
     influence of cortisone on radiosensitivity, 273  
     of oxygen on radiosensitivity, 214  
     protection of, 309  
     radiosensitivity of, 180  
 Lymphocytes, 286  
     destruction of, 328  
     disappearance of, 281  
 Lymphoid tumours, 304  
 Lymphomas, 330  
     effect of bone marrow homogenate, 339  
     induction of, 288  
 Lymphopenia, 328  
 Lysine, 302  
 Lysozyme, 139  
 Macromolecules, competition factor for radiomimetic chemicals, 196  
     effect of radiation on, 121  
 Maleic acid, 112  
 Malnutrition, produced by irradiation, 287  
 Malononitrile, 301, 303  
 Mammals, oxygen effect on, 213  
 Man, lethal x-ray dose, 220  
 Mass absorption coefficient, 6  
     variation with energy of radiation, 11  
 Massive irradiation, accidental, 347  
 Megakaryocytes, abnormal, 328  
 Meiosis, 159  
     chiasmata in pollen cells, 160 fig. 4  
 Melanin, 253  
*Melanoplus differentialis*, 257  
 Membranes, cellular, influence of radiation on, 185  
     of mitochondria, influence of radiation on, 185  
 Mercaptobenzothiazol, 294  
 Mercaptoethanol, protection of *E. coli*, 305  
 Mercaptoethylamine, see Cysteamine  
 Mercaptopropionic acid, 308  
 Mercaptopyruvate, 300  
 Mercaptosuccinate, 294, 300  
 Mercaptothiozaline, 294  
 Mercuribenzoate, 307  
 Mercuric chloride, 240

## SUBJECT INDEX

- Mesyloxy compounds, 192  
 clinical application of, 202  
 ring closure by, 193
- Metabolism, reduction of, 183
- Metabolites, relation to protection, 309
- Metaphase, 158
- Methaemoglobin-producing substances, protection by, 303
- Methane sulphonic acid esters, see Mesyloxy compounds
- Methanol, 148  
 changes in metabolism of, 232  
 decomposition of, 92
- Methionine, 238, 293, 302
- Methyl alcohol, see methanol
- Methyl linoleate, 301
- Methyl neostigmine, 288
- Methylamine, 302
- Microbiology, oxygen effect in, 210
- Micrococcus pyogenes*, 292
- Micronuclei, 162
- Micronucleus, 162 fig. 10
- Microsomes, 159, 187, 337
- Mineralo-corticoids, 268
- Mitochondria, 159, 185, 337  
 changes in enzyme activity of, 243  
 membrane, 185
- Mitosis, 157  
 arrest of in ascites tumours, 255  
 biochemistry of, 175  
 suppression of, 163  
 visible alterations produced by ir- radiation, 162 fig. 10
- Mitotic cycle, 160 fig. 1  
 poisons, 191  
 spindle, 160 fig. 5
- Molecular products, 98
- Molecular weight of macromolecules  
 from target size, 59  
 number average, 132  
 of polymers definition of, 132  
 viscosity average, 132
- Mollusca, 221
- Monkey, 220
- Monofunctional alkylating agents, see Radiomimetic chemicals
- Monotopic, 73
- Morphine, 304
- Monster, production of, 312
- Mosaic areas, 201
- Mouse, lethal  $\alpha$ -ray dose, 220  
 x-ray, 220
- neutron dose, 220
- RBE for killing, 75
- Mucosa, gastro-intestinal changes in, 287
- Mucous membrane, changes in, 285
- Multi-hit effect, 54  
 in terms of indirect action, 74  
 mechanism, 69
- Multi-target system, 58
- Mushrooms, 186
- Muscle, changes in cholinesterase, 256  
 incorporation of phosphorus by, 254  
 influence of radiation on contraction, 182
- Mustard gas, 190  
 inhibition of action by amines, 190  
 reaction with protein sulphhydryl groups, 197
- Mutagenic action, 156
- Mutagenic action and chromosome breaks, 169
- Mutagenic action, application of target theory to, 66  
 mosaic areas, 201  
 of radiomimetic chemicals, 190, 200  
 RBE of, 75  
 of sulphur mustards, 194  
 of x-rays, influence of oxygen on, 212
- Mutagenic effects, of atomic explosions, 353  
 protection against, 318
- Myclomatosis, multiple, 202
- Myleran, 202
- Myoglobin, 215
- Naphthalene, 128
- Naphthol, 128
- Naphthylamine, 128
- Naphthyl groups, energy transfer to, 129
- Nausea, 345
- Nephrectomized rats, 238
- Nephrectomy, 272
- Nervous system, sensitivity of, 348
- Neuro-blast, 348
- Neurones, 348
- Neutrons, 5  
 cataract production by, 353  
 effect on ascites tumours, 70  
 colloids, 117  
 lethal dose for mice, 220  
 mutagenic action of, 67

## SUBJECT INDEX

- oxygen effect with, 70  
 production of chromosome aberrations by, 71  
 reaction to give  $\alpha$ -particles, 3  
 transfer of energy by, 5  
 units of, 15  
 Neutrophils, changes in, 281  
 Newt, 220  
*Nicotana rustica*, 235  
 Nicotinamide, 54, 115  
 Nitrite, 303  
     oxidation of, 92  
 Nitrogen mustards, 192, 200, 201, 283  
     chromosome breaks by, 198  
     clinical application of, 202  
     development of resistance to, 201  
     protection by cysteine and cystamine, 298  
     reaction with DNA, 203  
 Nitrous acid, decomposition of, 38  
 Non-Newtonian viscosity, 132, 143  
 Noradrenaline, 215, 303, 311  
 Nuclear fragments, formation of, 162  
 Nuclear membrane, 157, 199  
 Nucleic acids, reaction with radiomimetic chemicals, 195  
     structure of, 142  
     see also DNA and RNA  
 Nucleoh, 158  
 Nucleolus, rupture of, 170  
 Nucleophilic alkylating agents, see Radiomimetic chemicals  
 Nucleoprotein, haematopoietic regeneration factor, 337  
     change in rigidity of, 152  
     effect of x-rays on, 152  
         irradiation *in vivo*, 153  
         splitting histone from, 152  
         staining of complex, 178  
         structure of, 144  
 Nucleotoxic effects, definition of, 202  
 Nucleus, 157, 160  
 Nucleus-cytoplasm, relative sensitivity of, 250  
 Nutrophils, changes in, 281  
 Octopus, 225  
 OH radicals, 82  
     competitions for, 315  
     distribution of in x-ray irradiation, 93  
         tracks of  $\alpha$ -particles, 93  
     formation chemical, 100  
     inactivation of enzymes by, 140  
     reactivity of, 99  
 OH<sub>2</sub> radicals, see HO<sub>2</sub> radicals  
 Oligodendroglia, 328  
 Onion, see *Allium cepa*, 161  
 Oocyte, 161  
 Organic substances, reaction of in water, 111  
 Orotic acid, 180, 244  
 Osmotic balance, disturbance in, 257  
 Ova, invertebrate, radioresistance of, 224  
 Oxalic acid, 112  
     decomposition of, 92  
 Oxidation-reduction reaction in irradiated water, 102  
 Oxidative phosphorylation, uncoupling of, 257  
 Oxygen, from decomposition of water, 79  
     combination with hydrogen by radon, 80  
     concentration in tissues, influence of cysteine on, 310  
     consumption by bone-marrow, 247  
         erythrocytes, 250  
         influence of radiation on, 229  
         liver homogenates, 251  
         potato tubers, 252  
     effect, 209  
         application to radiotherapy, 216  
         on deactivation of phage, 149  
         on decomposition of formic acid, 111  
     in degradation of DNA, 149  
     on enzyme inactivation, 140  
     evidence for indirect action *in vivo*, 65  
     on ferrous sulphate oxidation, 106  
     in microbiology, 210  
     with neutrons, 70  
         in radiochemical reactions, 106  
         and specific ionization, 106  
     influence of concentration of in radiochemical reaction, 106  
     poisoning, protection by cysteamine, 212, 298  
         similarities with radiation effects, 298  
 production of chromosome aberrations by, 212  
     breaks by, 169

## SUBJECT INDEX

- Oxygen—(Cont.)  
 tension, determination of in mammalian tissue, 214  
 in insects and radiosensitivity, 225
- Pair formation, 10  
 Pantetheine, 294, 296  
 Papaine, 240  
 p-aminobenzoic acid, 113, 115  
 Parabiosis, 333  
*Paramoecium*, 220  
 Pea, 72  
     protection by cyanide, 306  
 Penicillin, 285  
 Pentobarbital, 304  
 Perhydroxyl radicals, see HO<sub>2</sub> radicals  
 Permanent set, 135  
 Permeability, alteration of, 185  
     changes in red corpuscles, 286  
     increase in capillaries, 281  
     increase in lens, 353  
     intercellular, changes in, 253, 254  
 Peroxides, formation of in animals, 234  
     of fats, 233  
     influence of tocopherol on, 233, 234  
     mutagenic action of, 191  
     see also Hydrogen peroxide  
 Peroxy radicals, 312  
     see also HO<sub>2</sub> radicals  
 Pepsin, 60, 141  
 pH changes of in  $\alpha$ -tracks, 94  
 Phage, inactivation of, 149  
     target size, 58  
 Phenylalanine, 302  
 Phenylethylamine, 302  
 Phenol, 128  
     protection by, 301  
 Phosphatase, acid, 185  
     alkaline, 186, 251  
 Phosphate groups, esterification of by radiomimetic chemicals, 205  
 Phosphocreatine, 183  
 Phosphoglyceraldehyde dehydrogenase, 139  
 Phospholipids, 237  
 Phosphorus incorporation changed by irradiation, 254  
 Phosphorylation, coupled oxidative, interference with, 243 [243]  
 Photoelectric effect, 7  
     influence of wavelength of radiation, 8  
 Photoelectrons, 7  
 Photorestoration, 263  
 Phototropism, 223  
*Phycomyces blakesleeanus*, 220  
 Physiological chromosome effect, see under Chromosomes  
 Pig, 220  
 Pile irradiation, 4, 129  
     effect on polymers, 124  
*Pisum sativum*, 292  
 Pituitary, 306  
     adrenal reaction, 269  
     hormones, 267  
     role in radiation sickness, 267  
 Plasma, 245  
     changes in cholinesterase in, 257  
     fat content, 235  
     volume, 239  
     enzymatic changes in, 250, 253  
     proteins, changes in composition of, 238  
 Platelets, 286  
     changes produced by irradiation, 282  
     and haemorrhages, 283  
     transfusion, 284  
*Pneumococcus*, 177  
 Poison theory, 191  
 Polar bodies, 161  
 Pollen, irradiation when frozen, 65  
 Polonium, 3  
 Polyacrylates, 122  
 Polycythaemic rats, 341  
 Polycrylic, 124  
 Polyfunctional alkylating agents, see radiomimetic chemicals  
 Polyisobutylene, 62, 124, 126  
 Polymer 'bubbled', 123  
 Polymer protection, compared with protection of animals, 301, 314  
 Polymers, rendering infusible, 123  
     synthetic, 51  
     crosslinking of, 121  
     degradation of, 121  
         in aqueous solutions, 130  
         effect of radiation on, 121  
 Polymerization, 99, 116, 314, 315  
     of acetylene, 38  
 Polymethacrylic acid, 130, 213  
     degradation of in aqueous solution, 132  
     influence of oxygen concentration on degradation of, 106  
     protection of, 301, 314

## SUBJECT INDEX

- Polymethaphosphate, 130, 133  
Polymethylmethacrylate, 123, 125  
Polyplodity and radiosensitivity, 226  
Polystyrene, 124  
Polytetra-fluoroethylene, 125  
Polyvinyl alcohol, 71, 125, 130  
Porphyrin, 141  
Positron, 10  
Potato tubers, 186  
    blackening after irradiation, 252  
    oxygen consumption of, 252  
Potassium iodide, oxidation of, 81  
Potter-Elvchjem grinder, 337  
Prolactin, 267  
Prophase, 158  
Propionic acid, 112  
Prosthetic group, inactivation of in enzymes, 141  
Protamines, 145, 177, 205, 284  
    combination with DNA, 145  
Protected organism, behaviour of, 315  
Protection, chemical, 290  
    administration before and after irradiation, 305  
    of bacteria, 309  
    by blocking enzymes, 313  
    against cataract, 353  
    comparison of mouse with synthetic system, 301, 314  
    by competition, 312  
    competition for  $\text{HO}_2$  radical, 314  
    decreas of by anoxia, 310  
    diminution of primary effect by, 320  
    against direct action, 313  
    enhanced regeneration by, 320  
    and haematopoietic regeneration in factor, 341  
    and histological changes, 319  
        Krebs cycle, 309  
    metabolic changes, 309  
    methods of studying, 290  
    physicochemical mechanism of, 312  
    practical application, 321  
    reducing action and, 307  
    relation to toxicity, 306  
    by repair of macromolecule, 312  
    role of anoxia, 308  
    time of action, 241  
    by transfer, 313  
    universal nature, of, 305  
    vasoconstrictors, 311
- Protection, definition of, 305  
    of DNA against degradation, 148  
        *in vivo*, 153  
    against direct action, 127  
    evidence for indirect action *in vivo*, 66  
    by impurities, 52  
    of paraffin against crosslinking, 129  
    physical by lead, see Shielding  
        of polymers against crosslinking, 131  
    of proteins against indirect action, 137  
    relation to tissue anoxia, 215  
    by shielding, see Shielding  
    test for indirect action, 48  
    of viruses, 53
- Proteins, catabolism of, 231  
    changes in molecular weight on irradiation, 136  
    viscosity of, 137  
    as chromosome constituent, 176  
    combining with DNA, 205  
    degradation, protection against, 137  
    denaturation of, 137  
    direct action on, 134  
    dissociation by  $\alpha$ -rays, 135  
    inactivation by direct action, 134  
    influence of temperature on irradiation effects, 137  
    irradiation in aqueous solution, 136  
    mass absorption coefficient of, 11  
    metabolism, 237  
    nucleates, 144, 145  
        degradation of by x-rays, 147  
    plasma, changes in, 238  
    reaction with radiomimetic chemicals, 195  
    sulphydryl groups reaction with mustard gas, 197  
    u.v. absorption spectrum of, 138
- Protein synthesis, 175  
    role of DNA, 177  
    changes in ascites tumour, 255  
    role of heterochromatin, 179
- Proteolytic enzyme, 338
- Protons, 3, 13  
    breakage of chromosome by, 171  
    irradiation of cytoplasm by, 171  
    range of, 3  
    source of, 3  
    specific ionization density of, 21
- Prothrombin, 283

## SUBJECT INDEX

- Pteroylglutamic acid, 304  
Purine, incorporation into nucleic acid, 256  
Pycnosis, 170  
Pyrene, 128  
Pyrimidines, incorporation into nucleic acid, 256  
Pyruvate, 301, 309
- Quantum, definition of, 1  
relation to wavelength, 1  
Quinones, 186, 253
- Rabbit, irradiation of hind legs only, 328  
lethal x-ray dose, 220  
synthesis of haemoglobin in, 248
- Rad, 15
- Radiation-chemical yield, definition of, 36
- Radiation hazards of the population, 349
- Radiation injury of humans, 346
- Radiation sickness, 280, 333  
clinical symptoms of, 347  
cortical adrenal insufficiency in, 274  
first stage, 281  
late effects, 288  
second period, 283  
and stress, 267  
treatment of, 344
- Radiationless transitions, 33
- Radical formation in water, 82  
half life in tracks of ionization particles, 95  
hydration in liquids, 40  
rate of diffusion of, 95  
reaction with different substances, 96  
recombination, 92, 94, 96  
reactions in water, 99  
in liquids, 40  
spatial distribution, 93  
in water, 91
- Radishes, 251
- Radioactive materials, activity of, 16  
wastes, 350
- Radioactivity, natural, 351  
units of, 16
- Radiographic examination, 349
- Radiological examinations, 350
- Radiomimetic chemicals, 190  
biological action of, 197  
chemical reaction of, 192  
clinical application of, 202  
definition of, 190  
effect on cytoplasm, 190  
hazards of, 351  
mechanism of action of, 203  
reaction with acids and amines, 194  
DNA, 203  
need to be polyfunctional, 193
- Radioresistance, by sublethal irradiation, 271  
by cold, 183, 216
- Radioresistant complex, 313
- Radiosensitivity of chromosomes, influence of previous irradiation on, 165  
comparative, 220  
and effect of previous irradiation, 271  
increase of by Synkavit, 322  
influence of chronic anoxia, 342  
hypothyroidism, 308  
influence of polyploidy on, 226  
temperature on, 183, 216  
hydration, 72  
reason for variation of, 225  
and resistance to cyanide, 313  
variations of chromosomes, 173  
relation to DNA content of cell, 181  
relative of cytoplasm and nucleus, 191
- Radiotherapy, application of oxygen effect on, 216  
and chemotherapy, 357  
grid irradiation, 329  
possible role of protectors, 321  
usefulness of Synkavit, 322
- Radium, 3  
decomposition of water by, 79
- Radon, 3, 80
- Range of ionizing particles, 3  
in liquids and solids, 24
- Rat, changes in oxygen consumption, 229  
decrease of radiosensitivity by cold, 216  
lethal x-ray dose, 220
- Rat sarcoma, Jensen's, 257  
tumour, 198
- Rate of loss of energy, see Specific ionization

## SUBJECT INDEX

- RBE for cataract formation, 353  
 chromosome aberrations, 70  
     breaks, 173  
     definition of, 75  
     of density ionization radiation, 187  
     and indirect action, 74  
     summary of biological data, 75  
     and target theory, 57
- Reactivation of sulphhydryl enzymes, 140  
 Recessive lethals, 201 [140]
- Reciprocal influences, 328
- Recoil electrons, 8
- Recombination of radicals in liquids, 40
- Recovery, see Restoration
- Red corpuscles, permeability of, 286
- Redox potential, definition of, 101  
     of irradiated pure water, 101
- Reducing action of OH radicals, 100  
 substances, protection by, 303  
     relation to chemical protection, 307
- Regeneration, of haematopoietic organs, 316  
 influence of protectors on, 316  
 factor, from cell homogenates, 336  
     haematopoietic, 318  
     influence of enzymes on, 338  
     radiosensitivity of, 342  
     release of in the blood, 341  
     role in protection by chemicals, 341
- Relative biological effect, see RBE
- Renal failure, 238
- Repair of macromolecule by transfer agents, 313
- Replacement of cell constituents, 175
- Reptiles, radiosensitivity of, 221
- Resistance, development of in bacteria, 201  
     to radiation by anoxia, 209
- Respiration inhibition of and protection, 309
- Restitution of chromosome breaks, see Chromosome breaks
- Restoration after irradiation, 263  
     by storing at suboptimal temperature, 184, 264  
     of *E. coli*, effect of protectors on, 306
- Restoration factor, from spleen for bacteria, 265
- Reticulocytes, changes in haemoglobin synthesis in, 249  
 dog, 305
- Reticulo-endothelial system and restoration, 266
- Reticulosis, 344
- Retinal rods, 348
- Rhizopertha*, 223
- Riboflavin, 115
- Ribonuclease, 60, 139, 338
- Ribonucleic acid, see RNA
- RLE rate of loss of energy, see Specific ionization
- RNA, 142  
     degradation of by x-rays, 150  
     synthesis, 179, 253  
     changes in ascites tumours, 255
- Roentgen, definition of, 14  
     equivalent physical (rep), 15
- Rubber, 124
- Russula nigricans*, 186
- Sarcoma, Jensen's, 253, 257
- Sea-urchin, 224, 240  
 sperms, 72
- Secondary effect, 244  
 ionization, 93
- Seeds, influence of hydration, 72  
     irradiation when frozen, 65  
     radioresistance of, 224
- Selection natural, 351
- Selenite, oxidation of, 92
- Self protection, 50
- Semi-conductor, 43
- Sensitivity of cell, differences between chemicals and x-rays, 198  
     influence of oxygen tension, 209
- Sensitization by CO, 304  
     neighbouring irradiation, 329
- Serine, 309
- Serum albumin, 60, 136, 148
- Serum irradiated, effect on catalase, 257  
     haemoglobin synthesis, 249
- Sex-linked lethal mutation, influence of oxygen on, 213
- Sex-ratio, influence of atomic explosion on, 354
- Shielding, adrenals, 272  
     and haematopoietic regeneration, 334  
     liver, intestine and kidney, 332  
     of organs and limbs, 328  
     spleen, effect on survival, 330  
     thigh, influence on lymphoma, 330  
     variation in different strains, 331

## SUBJECT INDEX

- Silk worm, 183, 264  
 Single hit effect, 55  
 Single ionization effect, 55  
 Sister chromatids, 168  
     exchange of, 160 fig. 4  
*Staphilus*, 223  
 Snail, 220  
 Sodium azide, 301  
     changes in tissue concentration of, cyanide, 301 [274]  
     hydrosulphite, 309  
     salicylate, production of radioresistance by, 271  
 Soil, radioactivity of, 351  
 Solids, reactions in, 42  
 Somatic crossing-over, 201  
 Somato tropichormone, see STH  
 Soret band, 141  
 Spatial distribution of the ions formed by different radiations, 91  
 Specific ionization (ionization density), 19, 59  
     of  $\alpha$ -particles, 20  
     and breakdown of intracellular barriers, 76  
     of electrons, 21  
     influence on formation of molecular products, 88  
     oxidation of ferrous sulphate, 89  
     and mass and velocity of particle, 20, 21  
     produced by x- and  $\gamma$ -rays, 24  
     of protons, 21  
     relationship to voltage of therapy x-ray set, 26  
 Spectrum of radiation, 25  
 Spermatozoa, invertebrate, radioresistance of, 224  
 Spermheads, 147  
     from fish, 177  
 Spindle, 158, 162, 181  
 Spiraling, errors in, 162  
 Spleen, changes in haemoglobin synthesis, 247  
     enzymatic changes in, 243  
     erythropoietic activity of, 280  
     grafting after irradiation, 331  
     implantation, 266  
     in vivo activity, 247  
     pyknosis of, 319  
     regeneration of, 318  
     shielding of, 330  
     Spleen homogenates, active principle in, 338  
     regeneration by, 336  
     Splenic factor, restoration by, 265  
     Spondylitis, 344  
     Spores, bacterial, 65  
     Spurious breakage, 162  
     Staining, variation in nucleoprotein complex, 178  
     Steatosis, 237  
     STH, 267  
         protection by, 276  
     Sterility, 288  
         temporary, 351  
     Sterilization, protection against, 319  
     Steroid hormones, protection by, 304  
     Stickiness, 160 fig. 6, 162  
     Stimulating effects of x-rays, 246, 251  
     Stopping power, 13  
     Strain, differences and spleen shielding, 331  
     Streptomycin, 285  
     Stress, definition of, 267  
         role in radiation sickness, 267  
     Suboptimal temperature, storing at, 264  
     Succinate, 301, 309  
     Succinic acid, 109, 112  
     Succinodehydrogenase, 242  
     Succinoxidase, 242  
     Sugar, blood, 231  
     Sulphide, 293  
     Sulphoxide, 114  
     Sulphydryl compounds, effect of  $\gamma$ -radiation, 113  
         protection of mammals by, 294  
     Sulphydryl enzymes, inactivation of, 240  
         by indirect action, 140  
         reactivation of by glutathione, 140  
     Sulphydryl groups, oxidation of *in vivo*, 240  
         radiochemical reaction of, 141  
         reaction with radiomimetic chemicals, 196  
         role in chemical protection, 307  
     Sulphur, protection by, 308  
     Sulphur mustards, 192, 201  
         mutagenic action of, 194  
     Supercontraction, 135  
     Suprarenals, changes in, 275  
     Synkavit, 322

## SUBJECT INDEX

- Synthetic activity, increase after ir-radiation, 245
- Target size, associated volume method, 57  
calculation of, 56  
comparison with microscopic dimensions, 58  
influence of energy transfer, 130  
temperature on, 62  
and molecular weight, 59
- Target theory, 54  
application *in vivo*, 64  
criticism of, 61  
effect of energy transfer on, 62  
efficiency of different radiations, 56  
multiplicity of targets in organism, 58  
multi-hit, 54
- Taurine, 225
- Telophase, 158
- Temperature, influence on radiosensitivity, 62  
target size, 62  
lowering of and radiation effects, 183
- Template protein, 206
- Terramycin, 285
- Testis, concentration of protectors in, 318
- Testosterone, 304
- Tetany, hypocalcaemic, 239
- Tetraethylammonium, 288
- Tetrahymena geleii*, 256  
*pyriformis*, 233, 266
- Tetramethyl uric acid, 199
- Therapy x-ray set, specific ionization from, 25  
relation between voltage and wavelength, 2  
spectrum of radiation from, 25
- Thermal electron, 34, 82  
fate of, 85  
lifetime in water, 85, 103  
reaction of in water, 85, 103
- Thermoluminescence, 43
- Thiamine, 183
- Thigh, shielding of, 332
- Thioacetamide, 294
- Thioacetic acid, 294
- Thiobenzoic acid, 294
- Thioethanol, 294
- Thioglycolic acid, 308
- Thiosulphate, 303, 308
- Thiouracil, 308
- Thiourea, 113, 137, 148, 153, 308, 314
- Thiourea, tolyl, 128
- Thorn's test, 270
- Thrombopenia, 328
- Thymocytes, 305  
protection of, 310
- Thymus, atrophy of, 272  
cells, 170  
effect of thigh shielding on, 330  
pyknosis of, 319
- Thyroid, 308  
changes in after irradiation, 229  
iodine uptake of, 253
- Tissue anoxia, relation to chemical protection, 308  
mass absorption coefficient of, 11
- Tobacco mosaic virus, 52, 54  
ringspot virus, 60
- Tocopherol, 233, 234
- Toluene, 81
- Toluidine blue, 284
- Tortoise, 220
- Toxicity, influence on protection, 306
- Tracer experiments, see Isotope tracers
- Tradescantia*, 75, 168, 198, 210, 303  
mutations and oxygen effect, 67  
*paludosa*, 292  
*virginiana*, 160 fig. 2
- Transamination, disturbances in, 238
- Transfer agents and chemical protectors, 314  
influence on crosslinking, 130  
protection by, 313
- Transforming factor, 60, 177, 218
- Translocation, 167, 201
- Transphosphorylase, 183
- Trichinella spiralis*, 223
- Triethanolamine, 302
- Tri-ethylenemelamine, 202
- Trihydroxy-N-methylindol, 300
- Trillium erectum*, 161, 181
- Triphenyltetrazolium chloride, 304
- Tri-radial configuration, 167
- Triton, 166 fig. 15
- Trypsin, 60, 139, 141, 338
- Tryptamine, 302, 311, 314
- Tryptophane, 302
- Tryptophane peroxydase-oxydase system, 246
- Tuberculin, 284
- Tulipa fragrans*, 169

## SUBJECT INDEX

- Tumours, changes in spectrums of, 253  
concentration of amino acids by, 225  
in Synkavit, 322  
induction of, 288  
protection of, 318  
role of heterochromatin, 179  
sensitization of by cyanide, 293  
see also Cancer
- Tyramine, 302
- Tyrosinase, 252
- Tyrosine, 302
- Udotea*, 224
- Ultra-violet light, 100  
see also U.V.
- Units of radiation, 13
- Uracil, 180
- Uranium poisoning, 186
- Uranyl nitrate, 169
- Urea, changes in blood and urine content, 238
- Urethane, 199, 201, 202
- Uric acid retention, 238
- Urine, appearance of enzymes in, 186  
changes in, 272  
cysteamine metabolism, 299  
urea content of, 238
- U.V., absorption spectrum, of proteins, 138  
chromosome breaks by, 167  
difference from x-rays, 1  
light, 62, 100  
microscope, 179
- Vaccinia* virus, 58
- Vacuolization, 170
- Vascular fragility, 283  
system, 255
- Vasocostrictor, 311
- Vicia faba*, 75, 161, 198, 199, 304  
abnormal anaphases, 116  
DNA synthesis in, 181
- Victoreen dosimeter, 17
- Viscosity, non-Newtonian, 132
- Virus, chemical constitution of, 144  
efficiency of direct action, 53  
indirect action, 53  
influence of oxygen on radiosensitivity, 218  
protection of in solution, 53
- Vitamin antagonists, 191
- Vitamin A, 235
- Vitamin B, deficiency, 182  
group, protection by, 304
- Vitamin B<sub>12</sub>, 286, 304
- Vitamin E, see Tocopherol
- Visible mutations, 201
- Vomiting, 345
- W, see Energy to form an ion pair
- Walker carcinoma, 160 fig. 6, 162 fig. 9  
198 fig. 5
- Wasp, see *Habrobracon*
- Waste products, atomic, 350
- Water, decomposition by radium, 79  
excitation of, 83  
free radicals, production in, 81  
formation of H atoms in, 103  
hydrogen and oxygen by radon, 80  
ionization of, 82, 86  
mass absorption coefficient of, 11  
mechanism of radical formation in, 103  
proportion of forward and radical reaction, 89
- Water, pure, decomposition by radiation of different specific ionizations, 88  
formation of hydrogen peroxide from by x-rays, 87  
from by x-rays, 87  
molecular products from by x-rays, 87
- Wavelength, relation to energy of quantum, 1  
specific ionization, 24
- Weight loss, produced by irradiation, 287  
of mammals, 316  
influence of protectors, 316
- Wilson cloud chamber, 22, 93
- Wool fibres, 135
- x-rays, 1  
artificial radioactivity from, 5  
difference from u.v., 2  
effect of on water, 81  
from therapy set, 2, 25  
wavelength and voltage, 2
- x-ray irradiation, calculation of target size from, 57

#### SUBJECT INDEX

- Yeast, 69, 220, 226  
Yeast, radiosensitivity and poliploidy,  
    59  
    shape of survival curve, 59, 69  
Yield, of chemical reaction in water,  
    89  
    for formation of molecular products  
        from water, 90  
influence of specific ionization on, 89  
    substrate concentration on, 92, 96  
radiochemical comparison between  
    x- and  $\alpha$ -rays, 98  
    for enzyme inactivation, 139  
Zeta potential, influence of radiation  
    on, 117