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THE GROWTH PROCESS IN ANIMALS

"Read not to contradict and confute, nor to believe and take for granted . . . but to weigh and consider. . . ."

FRANCIS BACON

*Of Studies*

# THE GROWTH PROCESS IN ANIMALS

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## Preface

LIKE all scientific studies, that of growth in living organisms has become progressively more analytical, and increasingly concerned with what happens at the lower levels of magnitude. In the case of growth this systematic analysis began somewhat tardily, however, probably because growth was so very amenable to mathematical treatment from the outset, whereas in most subjects an exact quantitative treatment is the ultimate goal, achieved only as the culmination of the systematic analysis. Because growth studies began with relatively quantitative methods this tended to obscure the need for an orthodox analysis, starting from the qualitative and the descriptive. Very often growth was studied as a piece of pure mathematics, quite unrelated to flesh and blood. Consequently Morgan (1907) was very conscious of the great paucity of knowledge of any aspect of growth except its rate, and nearly 20 years later De Beer (1924) found the position still little changed.

In fact the tide of systematic analysis had begun to flow as long ago as 1899, as may be seen in the admirable treatment of the subject by Davenport, and by the year 1923 Brailsford Robertson was able to present a picture sufficiently clear in outline to fire a general desire to fill in further details. Robertson's book was outstanding also for its bold attempt to relate the results of the mathematical approach to date with those of anatomical, physiological and biochemical analysis; although this relation has not stood up to the test of subsequent thinking and research, the book succeeded in the wider aim of establishing a more balanced approach to the whole subject. It became clear that the mathematics of growth were not so very exact, that an orthodox analysis must be encouraged and that it must catch up with the mathematical approach before this could make much more headway; in consequence a number of new analytical disciplines developed very rapidly, particularly in the fields of cytology, bacteriology, endocrinology and nutrition. Later the availability of heavy and radioactive isotopes opened up *biosynthesis* as a vast new field of biochemical research. Other technical advances, including electron microscopy, facilitated research on the proliferation of viruses as a particularly instructive example of growth. Bacteriologists began to isolate and breed mutant strains, each lacking the ability to perform one particular step in biosynthesis; thus they were able to map biosynthetic pathways and at the same time they broached in earnest the great problem of the genetical control of growth.

With this sudden flood of information it has scarcely been possible, as yet, to take stock of the situation in the subject as a whole. Those actually borne on the flood of one of the new disciplines have scarcely the leisure to attempt a synopsis; in any case, the more successful their own field the less they feel the need to seek liaison with others. It is the general biologist who feels this need

most acutely and attempts to see the new fronts as extensions of the classical work of morphologists, histologists and embryologists. The present book aims at a synopsis of this kind, for the general biologist, though it is to be hoped that the specialist will find interest and help from the attempt to fit the results from his field into the plan of the subject as a whole. The further aim, in fact, has been to make a balanced assessment of the present position in the subject, with general conclusions which may serve as a basis for further work in the individual fields. The treatment therefore is not purely elementary: it assumes a knowledge of the elements and history of the subject. At the same time it should be well within the ability of the honours student in biology.

An attempt has also been made to see growth in its wider setting, as just one aspect of vital activities. At times in the past there has been a tendency to regard growth as something in a class apart from other aspects of physiology, a more pristine and fundamental property (Needham, 1959), but this is scarcely justified. In this work, where possible, the relationships between growth and other aspects of metabolism have been pointed out.

It is equally important to emphasize distinctions, and a particularly important one is that between growth and the other main component of development, differentiation. Technically the distinction is necessary as a preliminary to the specific analysis of growth. This does not necessarily imply that the two are completely distinct physiologically; there is necessarily considerable interdependence between them and one of the objects of the present analysis is to help in defining this more clearly. The definition is difficult, partly because embryologists have often made the simplifying assumption that they were dealing with differentiation alone, while workers in some of the newer disciplines have assumed that they were studying growth alone.

The separation of the present from the mathematical approach is largely a matter of topical necessity, and an ultimate welding of the two is to be expected. An indication of some of the points where they already impinge is given in the text. As an introduction to further literature in the mathematical field the reader is referred to Huxley (1932), Thompson (1942), Le Gros Clark and Medawar (1945), Brody (1945), von Bertalanffy (1960), Kavanau (1960) and Bonner (1961).

The book has been divided into two parts, a narration of the processes of growth as manifested at successive levels of magnitude, and the means by which these processes are controlled. In the first part the logical, historical order of analysis has been followed, from the highest to the lowest level of magnitude. In the control of growth it has been found most convenient to follow the sequence in the reverse order: consequently Part I is in the nature of an analysis and Part II of a resynthesis. The framework is one which should accommodate further developments in the subject.

The text has been supplemented with chemical formulae, equations and reaction sequences where these seemed essential for a full understanding of the text, but some which are very familiar, for instance the glycolytic sequence and

Krebs cycle, have been taken for granted. The same principle has been adopted for the pictorial illustrations, and a number of very obviously relevant pictures have been omitted because they are so familiar. An attempt has been made to offer mainly new illustrations or alternatives to those already available, those with a personal interest, and summarizing diagrams. The illustrations therefore are not intended to be comprehensive. I am greatly indebted to the following authors for permission to use their published illustrations for the figures indicated: Drs. H. Barnes and H. T. Powell (Fig. 25.1), Professor M. Calvin (Fig. 15.4), Dr. E. H. Cushing (Fig. 23.6), Drs. T. F. and N. I. Goreau (Fig. 4.10), H. K. Pusey, Esq. (Fig. 6.4), and Professor H. Selye (Figs. 23.1, 23.3, 23.4, 23.5, 23.7) and to the following editors and publishers of the works in which the illustrations were originally published: *Acta Endocrinologica* (Figs. 23.1, 23.3, 23.4, 23.5 and 23.7); The Company of Biologists (Fig. 6.4); Dr. D. P. Costello, Managing Editor of *Biological Bulletin, Woods Hole* (Fig. 4.10); Messrs. J. and A. Churchill (Fig. 23.6); J. B. Cragg, Esq., Editor of the *Journal of Animal Ecology* (Fig. 25.1); Dr. Dwight J. Ingle, Editor of *Perspectives in Biology and Medicine* (Fig. 15.4); and Dr. G. F. Stickley, Managing Editor of *Endocrinology*, J. B. Lippincott Co. (Fig. 23.2).

My sincere thanks are due to the Company of Biologists also for permission to use as Figures 3.5 and 23.9 previously published illustrations of my own. I am particularly grateful to Professor Selye and Dr. Barnes for lending me copies of their illustrations. To Professor T. Russell Fraser and Dr. Peter Curzen also I am extremely grateful for their generous help.

In order to keep the bibliography within reasonable bounds, it is designed as a key rather than a complete literature list. Where feasible, reference is made to good recent reviews of a particular field, rather than to the individual original papers. In some cases, however, it is necessary to refer to the latter and this reflects no discrimination against any papers not specifically mentioned. Usually the most recent contribution in a particular field is cited since this gives the key to the earlier literature, for those interested, and since this book is not primarily concerned with the historical background. No misattribution of priorities is intended by this.

I owe a long-standing debt to Sir Gavin de Beer as an inspiring teacher of this subject and to Sir Julian Huxley for the stimulus of his work and writings. To colleagues working in the field, and in particular to Professor P. B. Medawar, I owe a great deal of help and encouragement. In this book it is my hope to pass on these benefits, in some measure. My thanks are also due to those, in particular my wife and Mrs. J. A. Spokes, who helped in the task of typing the manuscript, and to Mr. P. L. Small and Mr. J. S. Haywood who prepared the photographs for many of the figures.

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## *List of Abbreviations used in the Text*

THIS does not include common chemical and physical formulae and symbols, or abbreviations used only once, and defined. Presuperscripts, e.g.  $^{32}\text{P}$ , indicate particular isotopes, usually radioactive ones.

Å	Ångström unit ( $10^{-7}$ mm)
a.a.	amino acid
a.a.a.	amino acid activating
a.b.	antibody
ACh	acetylcholine
ACTH	adrenocorticotropic hormone of the pituitary gland
ADP	adenosine diphosphate
a.g.	antigen
AMP	adenosine monophosphate, or adenylic acid
APGH	anterior pituitary growth hormone, or somatotropin
-ase	indicates enzyme hydrolysing the substance in question
asp . NH <sub>2</sub>	asparagine
ATP	adenosine triphosphate, or adenyl pyrophosphate
C <sub>1</sub> , C <sub>2</sub> , . . .	compounds with 1,2, . . . carbon atoms in the molecule
CDP	cytidine diphosphate
CMP	cytidine monophosphate or cytidylic acid
C.N.S.	central nervous system
CoA	co-enzyme A, co-transacetylase
CoF	co-enzyme F, co-transformylase
CTP	cytidine triphosphate or cytidyl pyrophosphate
D-	optically active compounds based on dextrorotatory glyceroce
DAMP	deoxyadenosine monophosphate, or deoxyadenylic acid
DNA	deoxyribonucleic acid
DNP	dinitrophenol
DN proteins	deoxyribonucleoproteins
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
GA	glutamic acid
GDH	growth and differentiation hormone of insects (ecdysone)
GDP	guanosine diphosphate
GN	glutamine
glu.NH <sub>2</sub>	glutamine
GMP	guanosine monophosphate or guanylic acid
GSH	glutathione
GTP	guanosine triphosphate or guanyl pyrophosphate

kcal	kilocalorie or Calorie (1000 calories)
$\alpha$ -KG	$\alpha$ -ketoglutaric acid or $\alpha$ -oxoglutaric acid
L-	optically active compounds based on laevorotatory glycerose
m.g.t.	temperature for maximal growth
MAH	moult-accelerating hormone of Crustacea
MIH	moult-inhibiting hormone of Crustacea
mV	millivolts
$m\mu$	$\mu/1000$ , or $10^{-6}$ mm
NA	nucleic acid
NAD	nicotinamide-adenine dinucleotide
NADP	nicotinamide-adenine dinucleotide phosphate
N/C	ratio of volume of nucleus to cytoplasm
NP	nucleoprotein
NTP	nucleotide triphosphate
$\sim P$	phosphate bond with energy transfer value of 8000 calories per mole, or more
PGA	pteroylglutamic acid
P <sub>i</sub>	inorganic phosphate, usually orthophosphate
P-lipid	phospholipid
PP	pyrophosphate
PRPP	s'-phosphoribosyl-1'-pyrophosphate
Q <sub>10</sub>	van't Hoff's temperature coefficient (ratio of rates of a particular process at temperatures 10°C apart)
RNA	ribonucleic acid
RNP	ribonucleoprotein
rH	$1/\log$ concentration of hydrogen atoms, in atmospheres
SH	sulphydryl radical
—S—S—	disulphur bond
T	temperature
t	time
TCA	tricarboxylic acid (Krebs) cycle
TDP	thymidine diphosphate
TMP	thymidine monophosphate or thymidylic acid
TMV	tobacco mosaic virus
TPP	thymidine triphosphate or thymidyl pyrophosphate
UDP	uridine diphosphate
UMP	uridine monophosphate or uridylic acid
UTP	uridine triphosphate or uridyl pyrophosphate
u.v.	ultra-violet radiation
$\gamma$ or $\mu g$	microgram, $10^{-6}$ g
$\mu$	$1/1000$ mm ( $\mu$ is also used for the "thermal increment" or "temperature characteristic" of physiological responses)
$\mu A$	microampere ( $10^{-6}$ ampere)
$\sim$	any bond with a high energy-transfer value

## CHAPTER I

### *Introductory*

THE non-specialist has little difficulty in defining growth in living organisms: it is the increase in size and mass of the body or its parts, essentially a quantitative change. The early students were equally explicit; sixty years ago Davenport (1899, p. 281) emphasized that ". . . organic growth is increase in volume, . . . it is not differentiation," and he quoted T. H. Huxley's still earlier and terser definition "increase in size." T. H. Morgan (1907, p. 240) wrote "Growth and differentiation are . . . the two processes by which the embryo is transformed into the adult. . . . The most general definition of organic growth is that of increase in volume." Subsequent writers have usually made the same distinction, for instance De Beer (1924, p. 1): "Growing implies getting bigger and this increase in size is what should strictly be termed growth. . . . Growth alone cannot be responsible for the processes of development." This might be put in the form of an equation:  $\text{Growth} + \text{Differentiation} = \text{Development}$ . Other recent writers, including J. Needham (1942, p. 506) and J. T. Bonner (1952, p. 7), have maintained this distinction, but unnecessary seeds of confusion were sown by such definitions as those of Vines (1886, p. 291) ". . . a permanent change in form accompanied by an increase in bulk" and Sachs (1887, p. 404) ". . . eine mit Gestaltveränderung innig verknüpfte Volumenzunahme." Earlier still, Pfeffer (1881, p. 46) defined growth as "die gestaltliche Änderung im Protoplasma-Körper," with the emphasis entirely on qualitative change, though there would seem to be no justification for this in the word *Wachstum*, a fairly direct relative of *auxesis*. Unfortunately this type of confusion occasionally has been sustained by more recent writers: Robbins (1928) says ". . . this phase of growth is commonly called differentiation or development. . . ." This implies the equation:  $\text{Differentiation} (= \text{Development}) + (\text{Other phases}) = \text{Growth}$ , with the further uncertainty whether or no "phase" has a purely temporal connotation. Some have added the unnecessary confusion of baroque ornament: ". . . Growth is the coordinated expression of incremental and developmental factors and functions."

The confusion is particularly unfortunate because, as in any subject, there is plenty of genuine complexity, which necessitates considerable qualification of any basic definition. In the first place growth often depends on a number of associated processes such as cell division and cell movements, which themselves contribute nothing to synthesis and size increase. Secondly a variable and often large proportion of size increase is scarcely due to the synthesis of essential

biological materials. In the extreme case of the mammalian lung much of the later increase is due solely to progressive stretching of the organ, through distension with air (Short, 1952). In other instances it may be due to an increase in the amount of water, salts, fat or other relatively simple materials: these are sometimes significant components of the living system but in any case mere measurements may give little idea of what actually has increased. The difficulty of deciding what is a significant component is recognized by Young (1950, p. 6) in his definition: "Growth is the addition of material to that which is already organized into a living pattern." Even simple inorganic molecules, if they are part of an organized living system are significant components and their increase, in organized pattern, is true growth. Proteins are the most important components of the organized fabric but there are many others.

This definition has the further virtue that it does not postulate an inevitable increase in size, but covers a wider manifestation of growth. Material may be removed as rapidly as it is produced, for instance in the pancreas and other glands, and the organ may show no size increase over a long period of vigorous productive life. It is therefore a comprehensive definition, which recognizes the peculiarities of growth in living organisms yet remains as near as possible to the original intuitive definition. It envisages the repair and maintenance manifestations of growth, and most others.

The first essential is to distinguish growth, the quantitative aspect of development, from differentiation, the qualitative aspect, and this is not difficult in principle. The two show a considerable degree of independence in their course and control (Chapter 27) so that there is good justification for distinguishing growth and for studying it in isolation. Unfortunately closer examination shows the distinction to be less clear cut. A good deal of what is usually regarded as differentiation is due to differential growth, in which there may be no change whatever in the quality of the units responsible, but only in their number and arrangement. A population of identical cells may proliferate more rapidly in one direction than another and this causes a change in their pattern, and a change in shape at a higher level of magnitude. Similarly a multiplication of identical molecules, differentially in the different directions within the cell, could change the shape of the latter. What are essentially quantitative growth phenomena therefore come to produce a qualitative change at the next higher level of magnitude. Within the molecule, however, differential growth, for instance of one side-chain relative to another, would produce a qualitatively different molecule, with new properties, and this would be true differentiation, at that level of magnitude.

Another type of differential growth which may account for a good deal of what is normally included in differentiation is the differential rate of proliferation between two or more types of unit which are already qualitatively distinct. If one type of cell becomes relatively more numerous than a neighbouring type then the quality of the organ as a whole changes. Similarly at a lower level of magnitude one type of protein molecule in a cell may multiply more rapidly

than another. There might be no other significant change, yet at the next higher level a qualitative change would be registered, since the proportions of the cell constituents would have changed. In this case a qualitative change would be recorded also at the next lower level, in the sense that the amino acid composition of the proteins of the cell, collectively, would have changed.

Two aspects of differentiation must be recognized, the spatial aspect just considered, where one region becomes different from another, and the temporal aspect, when qualitative changes occur within a particular unit in the course of time. Some components of this temporal differentiation likewise may be due entirely to growth processes. Knowledge is scarcely adequate to say much about this at the cellular and higher levels of organization, but at the molecular level it is manifested as the progressive change in character of such molecules as the proteins actomyosin of muscle, keratin of the epidermis and haemoglobin of the blood. If the actual molecules changed, through the replacement of certain radicals by others, this would be one of the clearest cases of pure differentiation, but in most cases examined it seems that the whole population of molecules is progressively replaced by one of the new type, produced by a modification of the pathway of synthesis—that is by a growth process. The rate of destruction of molecules by wear and tear (p. 5) is probably always high enough to render direct transformation a minor component at the most, in any of these instances of molecular differentiation.

Other cases usually regarded as temporal differentiation no doubt are due to the establishment of completely new pathways of synthesis, or to new extensions of earlier pathways, so that again the change is essentially one of growth. Pigments (p. 245), which are particularly easy to study because they carry a visible label, often appear relatively late in development and the later stages of their synthesis, at least, must begin only then. Again, enzymes concerned with the definitive work functions appear only at the time of visible morphological differentiation (Løvtrup, 1959). Both would usually be regarded as ideal examples of pure differentiation, but in fact they are changes in the pattern of syntheses.

These considerations leave little which can be considered as pure differentiation except the rearrangements of parts at the various levels, what Dalq (1960) has aptly called *morphochoresis*. An instance of this is seen in the early development of the eggs of many animals. There are often gross movements of material before cleavage, and subsequently a clear and progressive segregation of materials into particular cells and regions. Simple segregation is not the only process involved but no doubt it makes a major contribution. From the study of enzymic activity (Hermann, 1959) it is clear that the segregation becomes progressively sharper with time. The microlheterogeneity of the egg is replaced by a more macroheterogeneity of the embryo and it is perhaps worth emphasizing that this is, therefore, an anti-entropic change in the sense of making larger local differences in the distribution of matter and energy. Both growth and differentiation, therefore, effect a reversal of entropy (p. 252), with the aid of their supply of energy.

It is necessary also to define the relationship between growth and other processes in the body, that is the work functions or orthodox physiological processes. There is very clear evidence that differentiation, judged by chemical or by morphological criteria, must reach a certain stage in an organ before its work function begins and that the latter improves progressively with differentiation. There is a roughly inverse correlation between the state of both and the rate of growth, though growth often continues for a long time after organs are fully functional; Huxley (1932) has called this auxano-differentiation because it increases the amount of material in the current advanced state of differentiation. It shows that the normal work function does not preclude further growth in an organ, and indeed there are aspects of growth intimately related to the active work function. The most important is *functional hypertrophy*, a tendency for an organ to grow in proportion to the demands for work made upon it. The response is, of course, a very useful biological adaptation and no doubt there has been natural selection in favour of the association between work and hypertrophy. A related response occurs when part of an organ, or one of a pair, is destroyed: the remaining part then shows *compensatory hypertrophy* and largely restores the size and work of the original organ(s). As in the situation which induces functional hypertrophy, there is an increase in the ratio of demand to supply. Regeneration is a special case, of replacement after accidental loss of part of an organ, or even of a major part of the body; in this case replacement may still be regarded as a compensatory growth, by the surviving portion.

There is also a reciprocal phenomenon of *disuse atrophy*, proportional to the degree of subnormality in the demand made upon an organ. This again is probably an acquired biological adaptation, economizing on unnecessary material, and on its maintenance cost (p. 254). The complete hypertrophy-atrophy mechanism thus produces and maintains material just sufficient to meet the demand on each organ. The normal size of the latter is an average value within its possible range of functional response. The auxano-differentiation phase of ontogenesis is probably to be regarded as the early and most dramatic stage of this functional response: if organs are inactivated then, they show little or no further growth, and remain subnormal. In the adult the response still may be remarkably powerful, in either direction, but since it is a typical manifestation of growth its power does decline with age (p. 26). The average size of the organ and the range within which hypertrophy-atrophy can operate are genetically determined (p. 385); they record the cumulative results of natural selection upon past generations of the species, for an optimal level and range of performance by the organ.

The relationship between the growth and the work function of an organ may be even more intimate than already implied, that is to say hypertrophy seems to be part of the actual recovery process in the organ, following a bout of activity. This activity itself is typically exergonic or energy dissipating, while recovery, like growth, is essentially endergonic. It seems a reasonable specula-

tion that after an increased demand recovery overshoots the mark, providing the necessary extra margin for functional hypertrophy; it may be significant that the nuclei of neurons enlarge during a bout of vigorous physical exercise (Chance, 1956). Reciprocally, when the demand is below a critical level recovery may undershoot, leading to some degree of atrophy. Overshoot is well known in some typical growth processes (Comfort, 1956), including some instances of regeneration.

A further probability is that the functioning of an organ usually involves a reversal of the later stages of the synthesis of its fabric, and the recovery after work a reiteration of these stages of synthesis. Growth and the work functions could be regarded as complementary halves of an endergonic-exergonic cycle and their interrelationship would be a very fundamental one. This seems a reasonably true picture, at least for the contraction cycle of muscle (p. 170).

On this view of the work functions we could further regard the recovery phase as one component of the maintenance type of growth, which continuously makes good any losses by wear and tear and keeps the weight of normal adult human beings extremely constant over many years of the most energetic phase of life. This maintenance growth, because of its quantitative precision, is perhaps even more remarkable than the prior phase of juvenile growth, with its continuous increase in size. The energy and material necessarily used up in the work functions may be regarded as part of wear and tear in the broadest sense. Living organisms are systems in dynamic and not static equilibrium, in point of fact open systems in a steady state (Prigogine, 1955; von Bertalanffy, 1949, 1960; Oparin, 1957), with anabolism exactly balancing catabolism; in this light the two are inseparable properties of living systems.

It is evident that there are two main components of catabolism (Needham, 1959), one involved in the regular endergonic-exergonic cycle of the work functions, and the other in the accidental losses by wear—fortuitous entropic errors of operation which make all processes, physical and biological, much less than one hundred per cent efficient (p. 253). Living systems may have their own sources of wear, in addition to those of simpler physical systems. It is a condition of survival that they shall be able to counteract all these errors: probably all have come to evoke the recovery-hypertrophy type of response.

Wear and tear at the cellular and tissue levels has been recognized for a long time. The rate of scarfing of the superficial epidermis of mammals and the rate of destruction of blood cells are very great, and that of the gut mucosa and other tissues also is considerable. The replacement of these losses is known as *physiological regeneration*, a term which stresses the normality and inevitability of the whole phenomenon. After accidents such as wounding and haemorrhage the restoration process is speeded up.

At the molecular level wear and tear was at one time thought to be very slight, as measured by the excretion of those nitrogenous substances, such as creatinine, which vary so little in amount from day to day. Since the use of labelled metabolites it has become evident that the endogenous component

of metabolism is much greater than the creatinine index implies, and that much of the food material flows through the fabric of the body, rather than past it as envisaged in the original conception of exogenous metabolism. The flow through may be quite automatic, since there is evidence that the "turnover" of the fabric increases spontaneously with increase in food intake. Moreover, it would appear to be more than adequate to make good wear and tear on any scale normally encountered (p. 190).

Some have debated whether a high rate of turnover occurs even in tissues which are neither growing nor engaged in such productive activities as synthesizing material for secretion. Any measurable turnover in these tissues is considered by them to be due to the proliferation of new cells to replace losses. There is a tendency to accept the phenomenon of wear at the cell level but to question it at the molecular level. In fact there are good records of wear at this level, particularly among enzyme molecules (Morel, 1941; Haldane, 1954; Pryor, 1955). There must be additional damage at lower levels due to normal functions at higher levels; the simple mechanical effects of our own body weight on our feet and seat must cause a continual destruction at the cellular and molecular levels. There are many other opportunities for this kind of damage; new ones appear with each evolutionary increase in size and complexity (Needham, 1959).

In a sense the growth of an individual is itself a process of replacement of wear—of the losses, through death, of the individuals of the previous generation. At all levels, therefore, living organisms are adapted to make good wear and tear, and this is growth in the broadest sense. Some non-living systems also grow, and others survive for long periods, but living organisms are unique in the dynamic nature of their mode of perpetuation, requiring a perpetual and rapid growth activity in one form or another. There is some justification, therefore, for the view of Lorrain Smith (1932) that the key to the problem of growth is an understanding of life itself, a view which echoes that of Claude Bernard (p. 442).

By virtue of the essentially endergonic nature of typical growth processes they were at one time thought to contravene the second law of thermodynamics (p. 3), building up local concentrations of energy-rich materials. According to the second law, in any closed system there is a tendency towards maximal entropy, towards the uniform dispersal of energy. It is now generally recognized, however, that since living organisms are open systems their local accumulations do not constitute such a contravention; they are only temporary and local delays in the normal process of entropy of solar energy, which flows steadily through the biomass in a constant direction. Some of the solar energy in fact is degraded, in the process of storing the remainder: as usual it is not one hundred per cent efficient. An instance of such degradation, but borrowed from subsequent metabolism, concerns the lactic acid formed by the partial (glycolytic) degradation of glucose; one-fifth of this is completely degraded in effecting the resynthesis of the remainder to glucose.

It is also worth emphasizing that, although in general growth depends on endergonic syntheses, not every synthesis of this kind leads to a gain of energy by the body as a whole. If two molecules which are already in the body are then conjugated there can be no absolute gain in energy, and in fact there will be a loss through the energy expended in effecting the reaction. It will be true growth, however, if there is a gain by the permanent fabric of the body from material in transit. To the biochemist the reaction is one of *biosynthesis*, whatever the circumstances, but it does not constitute growth unless there is a gain of material or energy by the essential fabric.

Another apparent anomaly is that there may be individual exergonic steps in a pathway of biosynthesis. Nevertheless, if the pathway as a whole is endergonic, it is generally accepted as a growth process. Exergonic steps probably come most frequently at the end of pathways of synthesis, and so produce stable final products. At a higher level of magnitude, energy-releasing processes of cell destruction and resorption play an important part in further growth (p. 77), particularly during metamorphosis (p. 68). Cell deaths occur at all stages of development (Glucksman, 1951). When growth is speeded, then any necessary processes of resorption must be promoted at the same time, for instance in remodelling bones and enlarging nerve foramina.

With this provisional delimitation of the scope of growth an attempt may be made to answer the two essential questions about it: what is it, and how does it operate? The two form the bases of the two parts of the book. Part I is a consideration of what may be observed of the growth processes at each level of magnitude, beginning at the highest level. It is therefore analytical in character and this is also the normal historical direction of approach. Part II deals with the way in which growth is controlled, beginning at the opposite end of the scale of magnitude; it is therefore synthetic in approach.



P A R T I

*The Analysis of Growth*



## CHAPTER 2

### *The Rate of Growth*

SINCE growth is essentially a quantitative phenomenon its rate is its most important property. This is found to vary enormously; Robbins (1928) cited the contrast between a Kudzu vine which grows fifty feet in one season and the dwarf ivy which grows only an inch. Bamboos are said to grow as much as twenty feet in three days (Thompson, 1942, p. 160). Among animals one might mention the foetus of the blue whale which reaches a weight of two tons or more during its gestation period of  $10\frac{3}{4}$  months (Laws, 1959) and its adult weight of over 100 tons in three to six years (Thompson, 1942, p. 204), whereas the European pond tortoise increases in length only an inch in eleven years. Man grows more slowly than most animals, taking about twenty years to reach a weight of 170 pounds, but even among the lower Metazoa, which generally grow rapidly, the turbellarian, *Thysanozoon*, requires a year to reach its maximal size of  $2\frac{1}{2}$  inches.

Growth is essentially multiplicative, not accretionary; as Medawar (1945, p. 166) has put it ". . . what results from biological growth is itself typically capable of growth." Consequently it is more realistic to compare multiplication rates than absolute increments, though the latter are a reasonable guide if we start in all cases from a single cell, the zygote, having a uniform order of size. A bacterium may duplicate its mass every twenty minutes under optimal conditions and a simple calculation will show that at the rate of compound interest characteristic of growth this could produce in a matter of days a mass greater than that of the whole universe! The average interphase cell of a metazoan may complete its actual growth in the same short time (Latarjet, 1952) though the complete cell generation is usually longer than this, ten hours or more. Bacteriophage viruses grow even more rapidly than bacteria, increasing their mass a hundred-fold within a particular four-minute period of their reproductive cycle (p. 153). Here, and in general, there are very powerful controls keeping the high potential proliferation rate within actual bounds. Measured rates are, therefore, rates of control rather than of intrinsic growth potency. Potentially, no living organism has any difficulty in making good its losses (p. 187): in practice its growth seems to be regulated to ensure a bare replacement, with some flexibility to meet emergencies. Overproduction is as dangerous as a dwindling population (Needham, 1959).

#### **2.1. Growth Curves and Equations**

If the multiplicative rate of growth were constant the graph of size or mass against time would be exponential. This is evident from a consideration of what

is called the specific growth rate, the rate per unit of material already present,  $1/x \cdot dx/dt$ , where  $x$  is the size and  $t$  is time. This is identical with  $d(\log x)/dt$ , and if it is constant,  $a$ , then on integration  $\log x = at + \log b$ , so that  $x = be^{at}$ ,  $\log b$  being the constant of integration.

Growth curves, that is graphs of size/time, in fact often are exponential under ideal conditions, as in the early stages of growth in most organisms, and

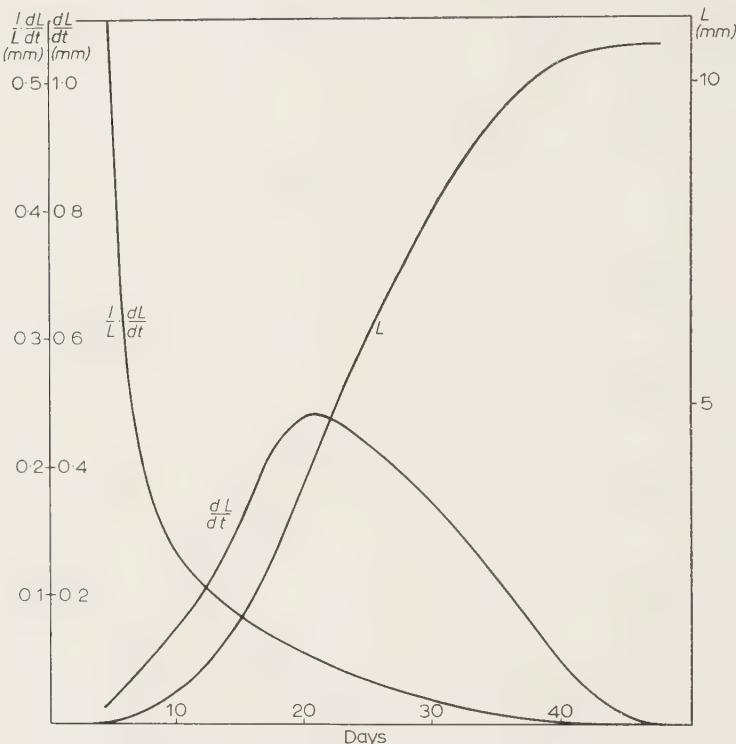


FIG. 2.1. TYPICAL GROWTH CURVE AND DERIVATIVES: REGENERATING POSTERIOR END OF EARTHWORM, *Eisenia*

$L$ , length of regenerate;  $dL/dt$ , first derivative, i.e. daily increment;  $1/L \cdot dL/dt$ , specific growth rate, i.e. daily increment per unit of existing length; all plotted as a function of time.

indefinitely in microorganisms under special experimental conditions (Novick and Szilard, 1954). The curve is one of increasing steepness, which is indicated by the equation to its slope,  $dx/dt$ . This is also the gross growth rate, so that total size increases with continuous acceleration.

Typically, however, the specific growth rate is not constant but is maximal at, or soon after, the outset (Medawar, 1945), and declines progressively to virtual or actual zero. This led Minot (1908) to regard the process of senescence,

which he equated with the decrease in specific growth rate, as also beginning at the outset, so lending colour to such ideas as those of the poets Edward Young (*Night Thoughts*) ". . . our birth is nothing but our death begun . . .," and George Peele (*Polyhymnion*) ". . . Youth waneth by increasing. . . ." The tissues of older individuals become less resistant to any inhibition of their growth (Medawar, 1940) and this may largely determine the fall in specific growth rate, *in vivo*.

The effect of the decline in specific rate on the simple size/time curve is not at first very great so that it still approximates to an exponential curve. As the effect increases the slope of the curve passes through an inflexion and decreases progressively towards an asymptote of zero slope, parallel to the time axis, and so to an upper limiting size (Fig. 2.1). The graph of its slope,  $dx/dt$ , against time (the first derivative of size/time) therefore is roughly bell-shaped (Fig. 2.1) but, as shown, often far from symmetrical (Robertson, 1923; Medawar, 1945). This asymmetry may be taken to mean that there is no close or absolute relationship between the cause of the initial high growth rate and that of the subsequent retardation, and this is what might be anticipated if, for instance, the one is an intrinsic potentiality and the other an imposed control. The curve of specific growth rate against time (Fig. 2.1) approximates to the exponential, with a negative exponent: it has initially a steep decline, becoming progressively less steep and tending asymptotically to a constant low value. Minot therefore contended that paradoxically ageing is most rapid at the outset! However this is perhaps the best reason to question his initial premise (p. 25).

Many different algebraical functions have been fitted empirically to records of growth; others have been derived deductively and then shown to fit actual data with some success. All recognize the phases of increasing and decreasing gross growth rate and some carry more specific implications about the nature of the complete growth cycle. The potentialities of this method of analysis have been discussed very thoroughly during the past thirty years (see, for instance, Gray, 1929; *Cold Springs Harbor Symposium*, 2, 1934; Le Gros Clark and Medawar, 1945; Baas Becking, 1945; *Proc. Roy. Soc. B.* 137 (1950); von Bertalanffy, 1960). The main conclusion is that measurements are rarely sufficiently precise to distinguish between the possible alternative relations which could be fitted to them and that in any event the gross curve probably represents the summation of many contributory processes and only by chance approximates to some simple relation also with a single biological meaning. The apparent simplicity in some cases is not to be attributed, as Robertson (1923) so admirably attempted to show, to some "master reaction." In view of the asymmetry of the first derivative curve (Fig. 2.1) even the idea of a single master control is questionable. However, as Robertson pointed out, the speed of a process is the speed of the slowest component and this may effectively determine the growth curve.

Medawar (1945) selected two relations as most plausible biologically and most useful empirically in fitting actual measurements, the logistic or

monomolecular-autocatalytic and the Gompertz. The former may be written—

$$x = a/(1 + be^{-kt}) \quad \text{or} \quad \log(a - x)/x = \log b - kt$$

where as usual  $x, t$  are the primary variables.

The Gompertz relation is—

$$x = ae^{-be^{-kt}} \quad \text{or} \quad \log \log a/x = \log b - kt$$

The logistic is an integration of the simple and theoretically satisfactory relation:  $dx/dt = k/a \cdot x(a - x)$ , which implies that the gross growth rate is the product of a factor which increases and one which decreases with the size of the growing structure,  $x$ . It recognizes the self-accelerating factor and also envisages the inhibitory factor as being size-dependent or auto-inhibitory. The specific growth rate is proportional to  $(a - x)$  which, as required, is an exponential function of  $t$ . The Gompertz relation, similarly, gives an exponentially declining specific growth rate, and there is some logical and experimental support for the function (Medawar, 1940). However, many sets of measurements do not closely fit either of these relations and there is none which commands universal approval for its wide empirical applicability and its theoretical appeal. For simple comparative purposes, without theoretical implications, the fitting of the best polynomial relation has been advocated (Needham, 1950; 1957), largely because it is mathematically easy to manipulate. Nelder (1961) has applied a generalization of the logistic equation, which includes the exponential, Gompertz and logistic relations as special cases. In a sense this type of method is retrogressive since the object of mathematical analysis is to specify more precisely the relationship which defines the process under consideration.

On the combined scores of biological rationale, ability to fit actual results and abilities to confirm and predict other facts about growth and metabolism, the relation of von Bertalanffy (1960) is of outstanding value. It is  $dx/dt = \eta x^m - \kappa x^n$ , where  $\eta, \kappa, m$ , and  $n$  are parameters, the first term representing the sum of anabolic factors and the second of catabolic factors. If  $x$  represents body weight, then  $m, n$  are found to vary between  $2/3$  and unity, indicating a dependence of anabolism and catabolism on surface area or on volume, or both. In some cases there is independent evidence on the precise dependence of each and this is found to agree with the values derived from data on growth. The relation predicts the equifinality of growth (p. 444), the absence of an inflexion in the growth curves of some linear measurements, and other features. It is consistent with a similar relation for the growth of individual organs, and with the allometry relation between organs (p. 35), and it predicts the differential nature of degrowth (p. 29).

In the special case of the regeneration of limbs in some of the Crustacea (Paulain, 1938) the length/time curve is virtually linear, gross growth-rate being virtually constant throughout. However, this is not common, even in

regeneration (Fig. 2.1). In the crustacean limb a blastema of cells is rapidly marshallled from a large and vigorous body, and a wave of proliferation sweeps in linear fashion along its length. This is one example of a particular pattern of growth; the more important of these patterns will be considered in the next three chapters.

## CHAPTER 3

### *Temporal Patterns of Growth*

THE typical and main temporal pattern has been considered in Chapter 2. Both gross and specific growth rates vary in a characteristic manner throughout the growth cycle; the sigmoid curve of size/time is the ideal curve and in some cases there may be only one such cycle during development. In other animals, however (see Brody, 1945), it is possible to recognize three or even four successive cycles, each giving a sigmoid curve. It has been pointed out that a relatively trivial factor, biologically, could induce an extra cycle in the growth curve, since a quite modest temporary retardation will automatically be followed by a return to normal, often reinforced by "rebound" (Wilson and Osbourn, 1960); the two phases together add a complete extra cycle to the curve. The transition from the first to the second cycle in man occurs around the time of birth, which possibly *is* a relatively trivial event biologically, and yet may be the cause of the inflexion in the curve. However, in some cases at least, the cycles are individually significant: the last in man is associated with puberty. Each of these is a unique phase of growth but in addition animals often have a number of rhythmically repeated cycles of growth.

#### **3.1. Growth Cycles and Rhythms**

Except in short-lived animals a seasonal cycle is superimposed on the main cycles. Both in poikilotherms and homiotherms there is usually maximal growth in spring, and often also a minor "autumn bite." The annual fluctuation may be slight in the tropics, so that it is undoubtedly due to external factors, for these show a marked seasonal cycle in higher latitudes; they usually act through food supply, ultimately (Gray and Setna, 1931). The arctic squirrel is adapted to grow very rapidly during its short summer season (Mayer and Roche, 1954).

A permanent record of seasonal growth remains in some structures, in the form of rings, layers, or concentric spheres. Those of mollusc shells, fish scales (Fig. 3.1), otoliths, baleen and others are regularly used to determine the age of the animal. In some cases there may be more than one visible change in texture per year so that the rings must be used with caution until the relation to season is established. All owe their clarity to a relatively simple spatial pattern of growth, restricted to the periphery.

Growth is usually inhibited during hibernation (Lyman and Chatfield, 1955). Consequently a hibernating rodent suffers no disability from its incisor teeth, which in normal times are persistently growing at a rate of 2 to 4 mm per

week (Sarnat and Hook, 1942) to balance the normally heavy wear. The diapause of insects (Lees, 1955) is a period of arrested growth, usually geared to season. In high latitudes the arrest is in winter, but for tropical insects it is usually in the dry season. Diapause is interesting in a number of ways, but particularly in the mechanisms of induction and termination or *breaking* of the

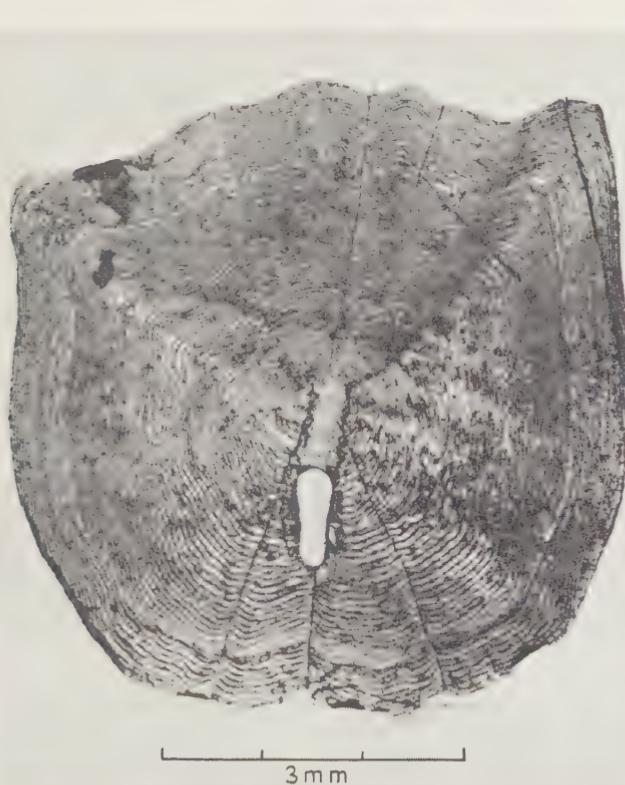


FIG. 3.1. SCALE OF TELEOST FISH TO SHOW CONCENTRIC RINGS OF GROWTH

This is a scale from the lateral line region and so has a hole and a tunnel near the centre for components of the lateral line canal.

(Slide: P. A. Trotman; photo: J. S. Haywood)

condition (p. 426). Among insects collectively it occurs at virtually every stage of the life cycle, though in the individuals of any one species, in one locality, the stage is usually very consistent (Andrewartha, 1952).

The plumage of birds and pelage of mammals often show a seasonal cycle of moulting and regrowth. That of cattle thickens in the autumn, through the elongation of some of the hairs, and these are shed in spring to give a thinner, cooler coat. Often there are two moults per year, for instance in the sub-arctic types which produce a white camouflage for the winter (Rothschild, 1942;

Hall, 1951). Light acting via the eyes and the nervous and endocrine systems controls the growth of the white hairs in autumn. These are then revealed suddenly by the rapid moult of the summer hairs. In boundary areas, such as Lincolnshire, the rate of the change depends on the variable experience of the individual in previous years. The spring moult is slower but continues, once evoked, even if the animal is re-exposed to winter conditions. The response thus is partly innate and partly imprinted by experience, but is also triggered off each year by the seasonal changes themselves. There are both spring and autumn moults also in many birds of temperate latitudes, usually in connexion with the breeding cycle. The more extensive autumn moult is followed by the growth of a less conspicuous "eclipse" plumage while the spring growth produces the bright colours of the breeding season, usually best developed in the male.

There are other seasonal growths associated with reproduction; one of the most striking examples is the growth of antlers in stags (Wislocki, 1956). In mature beasts an enormous spread of tines is produced each year. Visible growth begins in April or May and is complete by August. At one stage the branch of the trigeminal nerve which supplies the antler is growing as much as half an inch a day. The *velvet*, or skin, which covers the antler until it is fully grown, and carries the blood supply, is shed in September, apparently because the *burr*, or flange of bone which develops at the base, constricts the arteries and starves the tissues beyond. The rutting season is in autumn and the antlers are shed in mid-winter. Within a few months the whole cycle begins again. The tax on calcium and phosphate reserves must be very serious and the animals commonly gnaw the old shed antlers. The cycle is controlled by the same factors as the other reproductive activities.

Among the many seasonal growth processes connected with reproduction are the economically important ones, egg laying and milk production. There is often seasonal competition between reproductive and somatic growth, in animals which continue to grow after puberty. There is also seasonal competition between growth and the work functions; Fitt (1941) has shown that in children there tends to be a reciprocal relationship between the seasonal cycles for growth and those for other activities. A competition of this kind has been found in diurnal rhythms also.

The diurnal cycle is next in importance among natural growth rhythms. It is detectable in the rate of cell division (p. 338) which is maximal when the animal is resting, at night in diurnal animals such as man and during the morning in nocturnal animals (Bullough, 1949). Various other metabolic processes fit into the cycle, which is not so much a matter of competition between them as an economical sharing of energy during the twenty-four hours. The importance of rest for children is evident. The growth of regenerating structures also is subject to this cycle (Litwiller, 1940)—with the parallel lesson for the convalescent and the elderly! Productive activities such as spermatogenesis in the sparrow, also show this diurnal cycle, with maximal proliferation at night;

in this case, however, a low body temperature at that time may be more important than physical rest (Riley, 1937). The diurnal cycle seems to be absent from the hair buds, although present in the neighbouring epidermis (Bullough and Lawrence, 1958).

Many other kinds of growth cycle have been detected or suspected. In the growth of trees it is possible to recognize a cycle of approximately ten years, possibly correlated with the sunspot cycle, while in the long lived redwoods a longer periodicity of 150 years is detectable (Thompson, 1942, p. 240). The hair of rats grows for about 17 days and rests for a similar period; the mouse and other rodents have similar cycles. Human eyebrows grow for about eight and rest for twelve weeks. The mane of horses and some other hairs grow continuously so that there must be positive significance in the cases which are cyclic. Teeth grow in pulses with a period of 3 to 4 days, each producing one lamella. A cycle of one week in the deposition of bone has been described (Scissions, 1949) and a two-day cycle in some mammals (Davenport, 1899, p. 288) and in the chick embryo (J. Needham, 1931). Webster (1943) detected a curious periodicity of 7·6 months, or of multiples of this, in the recurrence of cancerous growths. There are probably a number of yet unrecognized rhythms in natural causative phenomena (F. A. Brown, 1957) including those due to the lunar day and month. School children show a growth rhythm correlated with that of the school terms (Allen, 1939), so that relatively artificial changes in external conditions may impose a growth rhythm.

Some of the cycles which have been recorded may be due to undetected rhythms in recording devices, particularly when the period is one of minutes or even seconds! The doubt is not so much that distinct events with such a speed should occur but that it was possible to record them with the apparatus available; much of interest may await refinements in recording. In some cases statistical devices may introduce spurious rhythms (Cole, 1957). There remain many which seem to show a genuine rhythm, often without any evident explanation at present. Some may reflect spontaneous metabolic rhythms of the particular animal. The particular length of the oestrus cycle in the different mammals has no very definite rationale: it is 3 to 4 days in the mouse, around 28 days in women, but longer or shorter in other primates, six months in the bitch, and so on. Some organs demand a fluctuating output of growth-promoting hormones (Biggers, *et al.*, 1957), or of other conditions (p. 418), and the growth itself is likely to oscillate in consequence.

A tendency for physiological processes to oscillate is probably the rule rather than the exception (F. A. Brown, 1957; von Bertalanffy, 1960). There must necessarily be two sets of factors, a promotor and a repressor set, so balanced as to maintain the process within its normal useful range. In order that the two may induce the kind of negative feedback essential for good control each usually evokes an increase in amount of the antagonistic set of controls. If there is an appreciable time lag at each step of the response this is very easily thrown into oscillations the period of which depends on the controlling agents rather

than on their subject matter. Oscillations develop in the growth of populations as well as in individual growth (Nicholson, 1950); in a confined space with plenty of food, fly larvae grow well and produce adults which lay many eggs. The crowding and competition between larvae of the following generations leads to poor growth, a lower percentage of emergence of adults, also with lower fecundity. Consequently, larvae of the subsequent generations are less

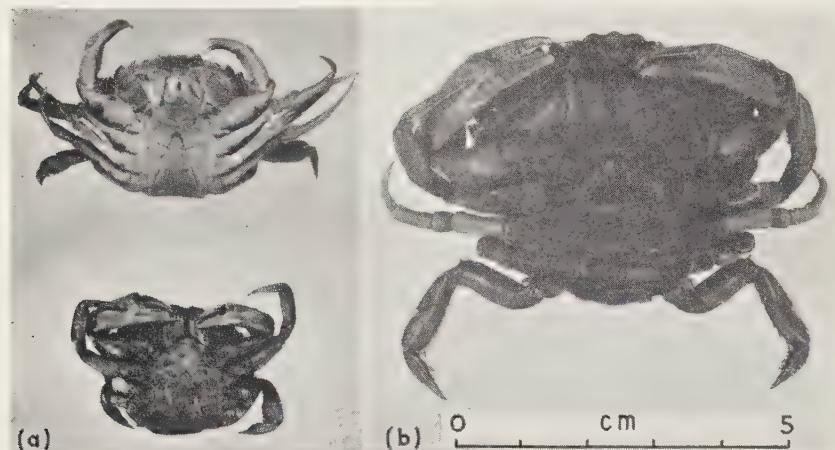


FIG. 3.2. GROWTH AND REGENERATIVE GROWTH IN THE SHORE CRAB,  
*Carcinus maenas*

(a) The smaller lower object is the exuvia (moult), from which the crab above had recently emerged. The general growth by inflation is evident. The regenerating third and fourth pairs of legs underwent a more extensive inflation, almost reaching their definitive size. (b) To illustrate the passive nature of the inflation of regenerating limbs. This crab died in the process of moulting. The exoskeleton covering the regeneration-buds of the third pair of legs was subsequently removed surgically, and the limbs promptly inflated. The buds of the fourth pair remain as controls.

(Photo: P. L. Small)

crowded and grow larger, so repeating the cycle. Competition between the adults also results in a fluctuating population. The numbers of lemmings and voles in the wild fluctuate with a periodicity of three to five years (Elton, 1942).

### 3.2. The Growth of Arthropods

In most arthropods size increase is periodic, the rigidity of the exoskeleton restricting it to the immediately postecdysal period, when the new cuticle is thin and extensible. The size/time curve therefore has the form of a staircase. However, there are some arthropods, including a number of parasitic members, with a permanently thin exoskeleton, and these show continuous size increase (Kükenthal, 1926-7; Wigglesworth, 1953). Regenerating limbs often enlarge continuously (Paulain, 1938), though in some of these there is also a dramatic eclosive enlargement after ecdysis (Fig. 3.2 (a)).

In the typical arthropod, which has its size increase limited to a brief phase of the moult cycle, the synthesis of new materials and cell division, also are periodic. However, they are not synchronous with size increase, which is due mainly to simple distension with water (Lowndes and Pannikar, 1941; Needham, 1946) or air. They are regarded as "true growth" and are often restricted to a short period in the middle of the stadium, maximally out of phase with the size increase in fact (Richards, 1951; O'Farrell and Stock, 1953). The harmonious regulation of true growth and size increase therefore presents an interesting problem. At one time it was thought that the aquatic arthropods simply absorbed water until the new materials were restored to a standard dilution, but, in fact, water intake is a triggered response (p. 425) and fails completely if synthesis in the previous stadium fails to reach a critical threshold level (Nouvel, 1934). Reciprocally, distension of the body with fluid may stimulate the next phase of growth: in the nymph of the bug, *Rhodnius*, distension of the body by a meal of blood causes tension in the epidermis which acts as a stimulus to further cell division (Wigglesworth, 1945); in this mechanism there is a lag of one instar before the response. The whole metabolism of arthropods shows cyclic fluctuations correlated with the moult cycle (p. 379).

Under good conditions a typical arthropod increases about twofold in volume at ecdysis, its linear measurements increasing by a factor of  $2^{1/3}$  ( $= 1.26$ ), the Brooks-Przibram-Dyar factor. It has, therefore, been suggested that there is precisely one division of each cell per instar, the appropriate amount of water to double the size being taken up after the next moult. However, in fact the increase is sometimes greater than this, but usually it is less; it decreases with age, in spite of the progressive increase in duration of the intermoult. Moreover, there is no good reason to believe that all cells proliferate synchronously, though a number may (O'Farrell and Stock, 1953). Further, in the later stages of development of most arthropods the growth of many tissues is entirely by cell *hypertrophy* and not by cell division or *hyperplasia* (Fig. 3.3).

Much of the size increase after moulting is due (Fig. 3.4) simply to the distension of the body spaces with water (Lowndes and Pannikar, 1941; Needham, 1946), but the cells themselves do enlarge to some extent (Needham, l.c.; J. D. Robertson, 1960). The second process may be regarded as restoring a standard dilution of the cell and the first as providing space for new cells. Osmotic forces are important as a proximate factor in the distension of aquatic arthropods, and in Crustacea there is an increase in osmotic pressure of the blood prior to ecdysis (Krogh, 1938; Robertson, 1960). This is due to another essential process, the removal of mineral from the old skeleton (Lowndes and Pannikar, l.c.), and it causes water uptake, so building up in turn an hydrostatic pressure. This is adequate to distend regeneration buds even of a dead *Carcinus* (Fig. 3.2. (b)) when the old exoskeleton is removed. The Isopoda moult in two stages, casting the posterior half of the exoskeleton first. Blood is then transferred to this half, distending it (Fig. 3.4) and at the same time decreasing the volume of the anterior half, so facilitating its withdrawal from the old shell

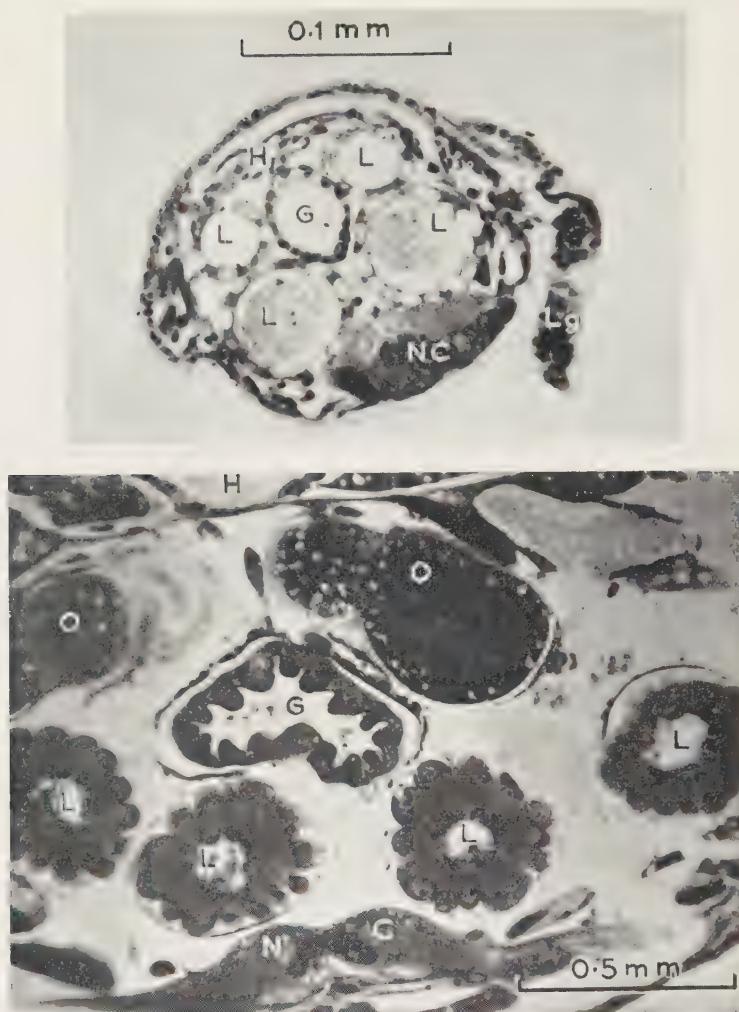


FIG. 3.3. TO SHOW GROWTH MAINLY BY CELL HYPERSTROPHY, IN THE GUT AND DIGESTIVE DIVERTICULA OF THE ISOPOD CRUSTACEAN, *Asellus aquaticus*

(*Above*) a transverse section of the body of a late embryo; (*below*) part of a T.S. of an adult, at a lower magnification. The number of cells in the sections of the gut and diverticula is virtually unchanged but they are much larger in the adult. The difference in cell shape is due merely to the lumen being distended with yolk in the embryo. G, gut; H, heart; L, digestive diverticula; Lg, walking leg; NC, ventral nerve cord; O, ovary.

(Photo: P. L. Small)

(Needham, 1946). Very striking changes result if the distension with fluid is not uniform but restricted to particular directions. Each time a female isopod crustacean lays a batch of eggs certain plates on the base of the thoracic limbs, the oostegites, expand greatly to form a brood pouch, and they regress just as

dramatically at the next moult when the brood is released. The expansion, restricted to the immediate post-ecdysal period when the exoskeleton is thin and elastic, appears to involve distension, with stretching, in length and breadth

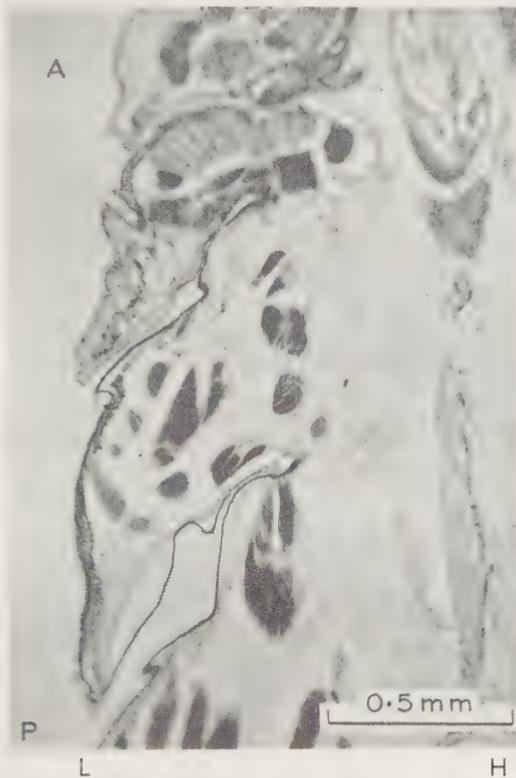


FIG. 3.4. TO ILLUSTRATE INTERNAL FEATURES OF GROWTH BY INFLATION, AFTER MOULTING, IN CRUSTACEA

This is part of a frontal section of the isopod crustacean, *Asellus*, which moults in two halves, the posterior (*P*) first. The section passes through the bases of two limbs in the anterior half of the body (*A*) and somewhat less than two in the posterior half. The latter have already grown by inflation of the haemocoelic spaces, and their new exoskeleton is developing. The anterior half also has moulted, but not yet inflated. *H*, heart; *L*, limb bases.

(Photo: P. L. Small)

only, the depth of the plate becoming less (Fig. 3.5). The consequent weakness is corrected by subsequent thickening of the exoskeleton.

Terrestrial arthropods are not able to use water in this way and often have a minimal amount of body fluid. They therefore distend the gut with air, so forcing the limited volume of blood into the organs which most need distension, such as the wing buds (Fig. 6.5). The mechanism is efficient enough: distension of the wings of some small insects is seen to be complete in a matter

of seconds. At the same time it is understandable that the nymph of *Rhodnius* should make good use of the distension caused by its meal.

Growth cycles geared to the periodic moulting of an exoskeleton occur also in nematodes, vertebrates and other groups. Snakes and many Amphibia cast

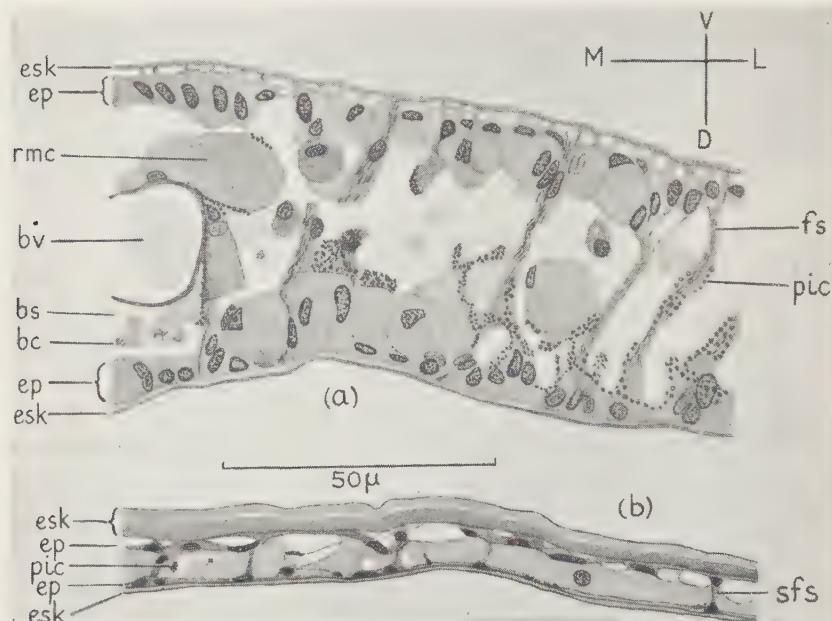


FIG. 3.5. PARTS OF DORSO-VENTRAL SECTIONS THROUGH OOSTEGITES, OR BROOD PLATES OF THE ISOPOD CRUSTACEAN, *Asellus*, (a) IN THE RETRACTED AND (b) IN THE EXPANDED CONDITION

Expansion involves an increase in superficial area at the expense of the dorso-ventral dimension, the individual cells becoming flattened in the process. Dorso-ventral fibres (*fs*) shorten. Vacuoles of material in the epidermal cells (*ep*) disappear and a thick exoskeleton is deposited ventrally. *bc*, blood corpuscles; *bs*, haemocoele; *bv*, afferent blood channel; *pic*, pigment cell.

(From Needham, 1942b, Quart. J. micr. Sci., 84.)

the superficial layers of their skin in a single piece but other reptiles shed it piecemeal, while that of mammals and birds is scarfed in small flakes.

### 3.3. Senescence and the Temporal Pattern

The relationship between senescence and the main temporal cycle of growth is of considerable importance to Man, both on his own account and as a stock-breeder. For the biologist it is of interest in connexion with the possibility that growth is just a part of an integral life programme which includes senescence. Growth powers of all kinds decline with the years (p. 4), while senescence increases: is the inverse correlation incidental to the life programme or is there a closer, causal connexion between them? Is the connexion as specific as

Minot believed (p. 13), so that the rate of decline in specific growth rate effectively measures the rate of senescence, or indeed *is* senescence? Senescence should then be most rapid at the outset and subsequently slow down at an exponential rate. If the process of senescence is related to the change in growth rate then its integral, the cumulative results of senescence, should be related to growth rate itself, and should be maximal when the growth and repair rate is zero (not of course when size increase stops (p. 5)). Unfortunately popular usage does not distinguish adequately between the process of senescence and the cumulative condition of senility: the latter increases to the end, just as size increases so long as there is a positive balance of growth over wear, but the rate may be maximal much earlier. In investigating the problem it is necessary to define senescence in a way which does not prejudge the issue: we may be permitted to call an animal or tissue youthful because its growth rate is high but until proved we must not call it senile if its growth rate is low. There are in fact plenty of independent criteria of senescence, and all may be regarded as components of a general decline in vitality and viability. The problem, therefore, is whether vitality and viability decline in close association with the general power of growth.

It should be pointed out that growth power itself does not always decline with age: in the skin (Cooper, 1952) and in some other tissues (Korenchevsky, *et al.*, 1950; Andrew, 1956) cell proliferation may accelerate in old age. This possibly might be a change akin to cancer (p. 95), which is most common in the middle-aged and elderly, and no doubt it is atypical. At the same time it is a warning against assuming a perfect inverse correlation between years and growth rate, with the further implication that any other property closely "tied" to age is necessarily also tied to growth rate.

There is some positive evidence in favour of Minot's view. For instance, basal metabolic rate per unit area or per unit weight appears to decline from an early age, and to decline exponentially, like the specific growth rate, most rapidly at first (Brody, 1945). This is true of a number of other properties including viability ("vitality"), measured as the reciprocal of the specific death rate (Brody, *l.c.*, Fig. 18.22). It is worth noting, however, that the specific death rate itself (Benjamin, 1957) gives a climbing exponential curve, convex to the time axis, implying that senescence rate is *minimal* at first and increases progressively, as most of us intuitively suppose (Needham, 1961). Minot, in effect, was assuming that senescence is proportional to the negative, and not to the reciprocal, of specific growth rate. A reciprocal relation not only best fits the quantitative data available, but seems logically preferable, and it removes Minot's grotesque paradox.

It also helps to remove the difficulty that individuals are manifestly in their prime between puberty and middle age. Immunological efficiency, healing ability and other properties are maximal at puberty while others are maximal at various times during the next two decades, in man. Senescence increases so slowly during all this time that quite minor contributory factors may materially

shift the age of maximal performance. Reproductive maturation and growth may be one such factor which has shifted the maximum for a number of properties to a more advanced age than originally, at the age of maximal specific growth rate. There is considerable variation also in the age of maximal sensitivity to different diseases (Dublin, 1952), and this may reflect the same general phenomenon. Pneumonia affects the very young and the very old and there are others which likewise reinforce the idea of a prime of life much later than the age of maximal growth and much more protracted. It seems likely that the state of senility is the reciprocal of this condition, which may be looked on as the summation of performance in all these properties, and not in growth alone. If the senile state is minimal during the prime then, it must be greater in the very young as well as in the old, and this may underlie the popular recognition of old age as a second childhood: the paradox is at least as acceptable as Minot's.

The view that vitality is the summation of performance in all vital activities and properties, is supported by positive evidence that all are integrated physiologically. The condition of hybrid vigour (p. 386) involves a high growth rate, a large adult size, longevity, productivity and other properties, all improving viability. Lancing (1947), by consistently selecting the offspring of young (parthenogenetic) rotifers for breeding further generations, produced *pediaclones* which grew slowly, produced many offspring and were long-lived. Reciprocally, by selecting always the offspring of old mothers he produced *geriaclones* with the contrasting syndrome. In other cases a relationship simply between growth and senescence has been recorded, but it does not follow that the syndrome was limited to these properties alone. In the pituitary condition known as *progeria*, for instance (Fig. 23.6), there is an arrest of growth and a remarkable acceleration of senescence. In Suctoria (Rudzinska, 1952), Cladocera (Ingle, 1933) and other animals (Sinclair, 1955), intensive feeding accelerates growth and maturity and shortens the life, while underfeeding rats (McCay *et al.*, 1943) slows their growth and prolongs their life.

In a number of these instances a high growth rate is correlated, as expected, with longevity but in Lancing's results and in others the converse is seen, and presents a major problem. Comfort (1953) was unable to confirm Lancing's results, in a sexually reproducing animal and Fritsch (1956) failed even when using another parthenogenetic type, *Daphnia*. Again, there is no very good evidence that the acceleration of growth in children during recent generations is leading to earlier senescence. The feeding experiments seem well authenticated, however, and they all show the direct and not the inverse relationship between growth rate and senescence. It may be that environmental changes tend to produce this kind of response while genetic conditions, such as hybrid vigour show the inverse relation; progeria also is likely to be genetic in causation.

The general conclusion would seem to be that growth and senescence probably are related as components of an integrated life programme (Comfort, 1956), but that there is no unique or peculiar relationship between them. The

idea that animals are genetically determined to run a definite and integral life programme is supported by a good deal of evidence, such as the relative constancy of the maximal size, regardless of the rate at which it is attained (p. 444). Not only is the programme relatively constant among the individuals of the species but also between species, as in the energy requirement for doubling the weight (p. 256). It is an interesting fact that during its lifetime of three years the heart of the mouse beats about as many times ( $1 \cdot 1 \times 10^9$ ) as that of the elephant  $1 \cdot 0 \times 10^9$ ) in a life span of 70 years. Kuhn (1958) has suggested that an automatic increase in optical impurity of molecules may play a part in senescence, and there are other properties which might change automatically in this way.

A genetically determined programme is likely to be the outcome of evolutionary processes, and Medawar (1946) has outlined a natural selection theory of the origin of senescence. He reminds us that the phenomenon of senescence should not be taken for granted. Microorganisms are potentially immortal and so are metazoon cells isolated *in vitro*; there is presumably a biological reason for senescence, as there is also for the cessation of growth in the individuals of many species. Medawar points out that the reproductive value of an individual is maximal at puberty and that there will be maximal selection against any deleterious genic manifestations at that time, with progressively less selection against any appearing at greater ages. The reproductive value of an individual is virtually nil once individuals of the next generation reach puberty and there will be little selection against deleterious or senescent manifestations which appear after that time. Through selection acting on the relevant genes in various genotypic combinations there will also be a tendency for the time of first expression to be pushed onwards in life but to halt at the age when the individual becomes reproductively redundant. At this age senescence might be expected to accelerate considerably. In the wild, animals rarely live long enough to show much senescence, but man and domestic animals do, and their mortality curves reflect the feature. The mortality rate remains very low throughout the prime of life and then suddenly rises.

To some extent elderly individuals are a biological liability if the food they consume could have gone to support new individuals; in this event they may be said to depress the reproductive rate of the species and in consequence the number of new genetic experiments it can make. In Man this may be more than offset by the value of the experience of the elderly, but in most species there could be, with advantage, also a positive selection in favour of senescence and death after the age of reproductive redundancy, and not merely selection against it in earlier years. Species with a short life span and a high turnover rate of new individuals in general should prove to be genetically more plastic and adaptable than those with the opposite characteristics. In Man there appears to have been selection for longevity, so great has been the value of individual experience. At the same time there has been selection in favour of a menopause in the female: this frees her for other social duties and leaves childbearing to

younger women with an optimal internal environment. In all these speculations it is notable that the significance of senescence lies with reproduction rather than with growth.

There is an environmental contribution to senescence (H. B. Jones, 1956), and this is to be expected on Medawar's theory. The relaxation of counter-selection with increasing age will apply as much to any inadequate responses to environmental hazards as to more orthodox genetic disabilities. Recovery from the insults of accident and disease becomes increasingly incomplete with the advancing years and this in turn increases the liability to fall victim to further stresses. In the recipe for long life, therefore, discretion may be very much the better part of valour. Consequently there is reason to be sceptical of the records of the Dane, Christian Drakenberg, who is reputed to have enjoyed 146 years full of action, hardship and hazard, as soldier, sailor, slave and lover.

The process of recovery from stresses (Selye, 1950) is essentially one of the growth processes and its declining efficiency with age is perhaps one of the most evident of the direct relationships between senescence and a declining power of growth. Both are accelerated by environmental insults. At the same time it seems likely that a declining power of growth is also one of the major genetic disabilities which has been permitted to accumulate in those who are beyond the critical reproductive age. The phenomenon of cancer (p. 95) indicates that paradoxically an abnormally enhanced growth potency is another of these disabilities.

In conclusion it may be said that senescence is due to the failure of selection for viability after a critical reproductive age, or even to active counter selection. It is minimal in the prime of life and subsequently increases progressively in rate. It is the resultant of all genetic disabilities and environmental insults. Genetic disabilities include errors of growth and of other properties contributing to the integral life programme. Since growth in the broadest sense accounts for so much of the life programme its decline inevitably is a major correlate of senescence.

### **3.4. Growth in Reverse**

One of the outstanding features of senescence is the general shrinkage of the body. In view of the integration of the whole life programme it is reasonable to regard this as the continuation of the decline in net growth rate, through zero in the prime of life to a negative value (Needham, 1961). The balance between wear and replacement now tilts in favour of catabolism. Not all degrowth in mammals is of this senescent type: the smooth muscle cells of the uterus regress dramatically after parturition, as a normal physiological event, and hypertrophy once more during the next pregnancy (p. 119). There are other examples, grading through disuse atrophy (p. 4) to the senescent and frankly pathological types. Another physiological type of degrowth is associated with metamorphosis (p. 68) and with other more restricted cases of remodelling, as in the head of growing long bones (p. 77).

In the lower animals negative growth or degrowth is commonly reversible, sometimes to a remarkable degree. It is a normal response to starvation and to some other conditions. It involves dedifferentiation also, an indication that the two components of development are directly rather than inversely related (p. 435). In starved *Paramecium*, cilia and other specialized structures are dedifferentiated as the animal grows smaller (Wichterman, 1953). The ascidian, *Clavellina*, degrows to a minute sac of cells (Huxley, 1921) and equally striking powers are shown by some coelenterate medusae (De Beer, 1924) and by the nemertine worm, *Lineus* (Dawyoff, 1924).

The dedevelopment appears to be in many respects a direct reversal of normal development. The medusa, *Chrysaora*, may regress to a gastrula-like body and *Lineus* even to a blastula. Perhaps this is some further justification, therefore, for the popular belief that the age degrowth of Man is likewise a partial return to second childhood (p. 26). The reversal is not limited to growth proper, but also affects other physiological processes. There are severe limits to the degree of reversal possible in mammals and there are some limits even in lower animals (Abeloos, 1928; J. Needham, 1942). Starved planarians do not fully recover the high growth rate of embryos and so could not become immortal by this means: presumably the phenomenon of irreversible senescence occurs even in animals as lowly as this. As in young animals living on a restricted diet which permits little growth (p. 444), the potential growth rate proves more dependent on size than on age; the latter has some effect, however, which is irreversible and no doubt is a major expression of senescence. This is why irreversibility is so pronounced in the mammals; even in this group, however, growth rate shows a high degree of size dependence (McCay, 1952).

Differentiation also is much less easily reversible in mammals than in the lower animals. This is evident both in senescent degrowth and in the response to starvation. Sometimes there seems to be an actual increase in differentiation (Robertson, 1923) because of the differentially greater rate of degrowth. This may be seen even in animals as primitive as starfish, which become more deeply pigmented when fasting (Vevers, 1949).

There is another reason why differentiation may appear to increase during the regression of starvation: the various organs degrow differentially, in general proportionally to their dispensability (Huxley, 1932, p. 201). The nervous system and heart are particularly important and are very resistant to degrowth. Robertson (1923) gave the following percentage losses in a starving cat: nervous system and heart 3, bones 14, pancreas 17, gut and lungs 18, skin 21, kidneys 26, muscles 31, testes 40, liver, 54, spleen 67, fat 97. *Paramecium* shrinks more in girth than in length and its spiral shape therefore becomes more pronounced. The increase in pigmentation is uniform throughout the integument of the starfish (Vevers, l.c.) but this may be regarded as a single organ.

During degrowth there is increased hydration. This might be simply filling spaces vacated by other materials but, on the other hand, it may be one of the indices of a reversal of development (p. 266). The individual tissues

regress and there is some tendency towards reversible syncytium formation (Robertson, *i.c.*). While some of the changes of degrowth perhaps could be regarded as rejuvenation this could scarcely be claimed for syncytium formation. In general the cells become smaller and fewer, though in hibernating frogs the size alone is said to decrease (Robertson, *i.c.*) and in starving planarians only the number (Hyman, 1951, Vol. II, p. 206). In the salamander the number may actually increase: certainly the number per unit volume is likely to be greater when their size decreases. Since in general the cells of embryos are larger than those of adults, in this respect also there is no simple rejuvenation. The cytoplasm shrinks more than the nucleus, probably because it is more dispensable, but this increases the nucleo-cytoplasmic ratio (p. 343) and might be regarded as a rejuvenation change. Atrophy is differential also among the various enzymes of the cell, again perhaps depending on their relative dispensability.

The graph of size against time for the phase of senescent shrinkage in man is sigmoid (Needham, 1962), since the rate at first accelerates and then slows down. This form is perhaps to be expected on Medawar's theory (p. 27), of a procession in the time of expression of senescent features to just beyond the reproductive age but not necessarily further. The degrowth of individual organs, such as the thymus (Fig. 4.1), follows the same type of curve. This probably implies that, as again in the dwindling of an old population of micro-organisms (p. 113), degrowth is not simply a failure of the balance of anabolism over catabolism but a well-controlled process, just as much as the progressive phase of growth. This might therefore also be taken to support the idea (p. 27) of positive selection for senescence. T. B. Robertson (1923) fitted to the regressive phase an exponential relation:  $x = x_0(1 - a)^t$ , where  $x_0$  is the initial, maximal size. Pézard (1921) fitted a parabolic relation:  $x = x_0 + a(t_0 - t)^2$ ; neither, therefore, recognizes the final phase of retarded shrinkage, giving the curve an inflexion.

The general conclusion is that degrowth can be extensive, particularly in the lower Metazoa, and that it is to some extent a direct reversal of growth. It is not an indiscriminate emaciation but has the adaptive feature of sparing the most important organs and tissues. It has a sigmoid progress curve and shows other evidence of being a well-controlled process. For special purposes it occurs in particular organs even of well-nourished animals.

## CHAPTER 4

### *Spatial Patterns of Growth*

IN considering the absolute growth of an organ or animal it is assumed for convenience that the growth rate is uniform all through and that there is no spatial "pattern" of growth. This simplicity rarely holds in practice. The

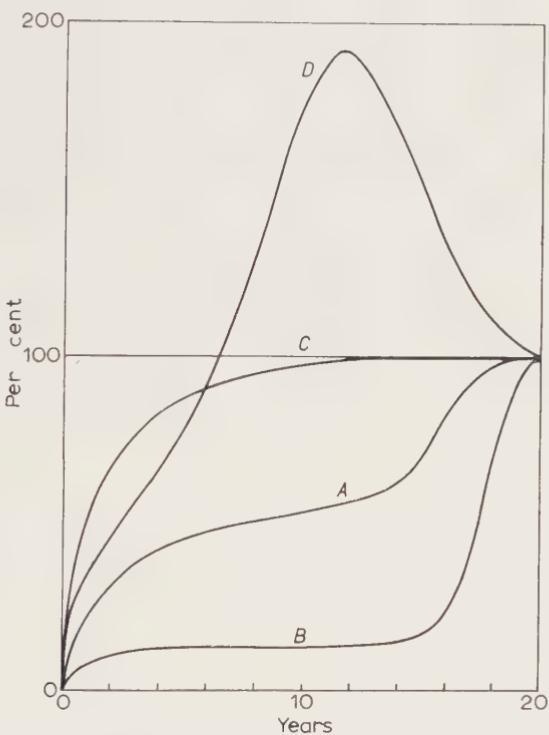


FIG. 4.1. GRAPHS OF SIZE, AS A PERCENTAGE OF ADULT SIZE, AGAINST AGE, IN MAN  
(Based on the curves of Scammon)

A, the whole body; B, the reproductive organs; C, the brain; D, the thymus.

growth curves of the various organs differ in shape, time scale and other features (Fig. 4.1). Most animals change in shape as they grow and this usually involves at least some degree of spatially differential growth. Most animals have a relatively complex shape, by geometrical standards, and for mechanical reasons

they rarely could grow uniformly throughout, even if this were desirable for other reasons. In fact, even animals with a simple shape could not grow uniformly throughout without setting up strains which would cause distortions and shape changes, if not more serious effects. Paradoxically, therefore, uniform growth may cause a change in shape. The bodies of most animals are plastic but there are limits to the amount of distortion which they can tolerate. There are often physiological reasons why fortuitous shape changes due to

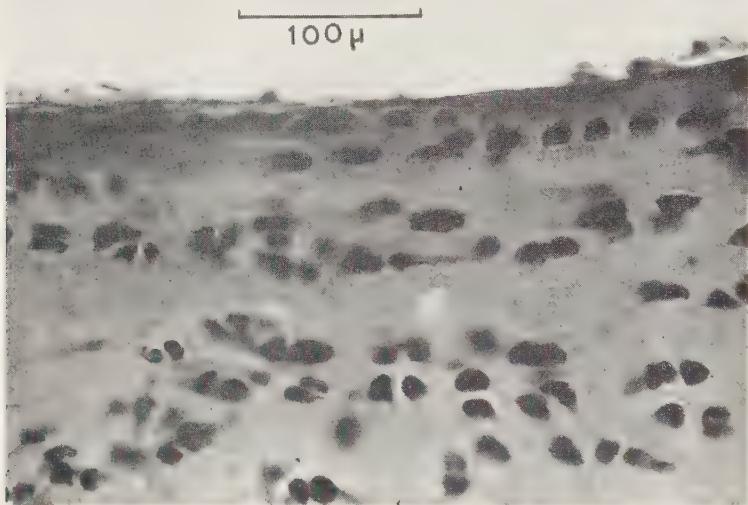


FIG. 4.2. SECTION OF CARTILAGE FROM THE CUTTLEFISH, *Sepia*

At the top is the edge of the cartilage, where perichondrial cells are becoming incorporated by the deposition of matrix round them. The cells are still in distinct layers parallel to the surface. Deeper in the matrix cells are in nests of two or four, owing to cell division after imprisonment in matrix. In contrast to vertebrate cartilage there are distinct cell processes running out into the matrix.

(Photo: P. L. Small)

growth could not be tolerated and, paradoxically again, differential growth is often necessary to preserve a required shape; a spherical shape is preserved most simply by growth which is differentially restricted to the periphery. Uniformly disseminated growth is not necessary to preserve shape and this relieves the problem of its actual impracticability.

Although completely uniform, disseminated growth is rare, some *intussusception* does occur, differentially distributed in patterns having varying degrees of regularity. It seems likely that for a time the growth of young cartilages approximates even to uniform dissemination. The cells initially enclosed in the extracellular matrix continue to divide and to produce more matrix. The extent of this is limited, however, and eventually nests of two or four cells are

seen imprisoned together in the same lacuna (Fig. 4.2). Henceforth growth is restricted to the periphery (p. 59).

The spatial patterns of growth no doubt are a compromise between the sanctions of growth itself and biological requirements for particular forms and structures. The latter are often the dominant factors and explain some of the more unexpected patterns of growth. Thus the abdomen of the female of *Pinnotheres*, the pea crab, has a point of maximal growth near the centre of the organ (Fig. 4.3), mechanically the least tolerable of patterns in a solid structure.

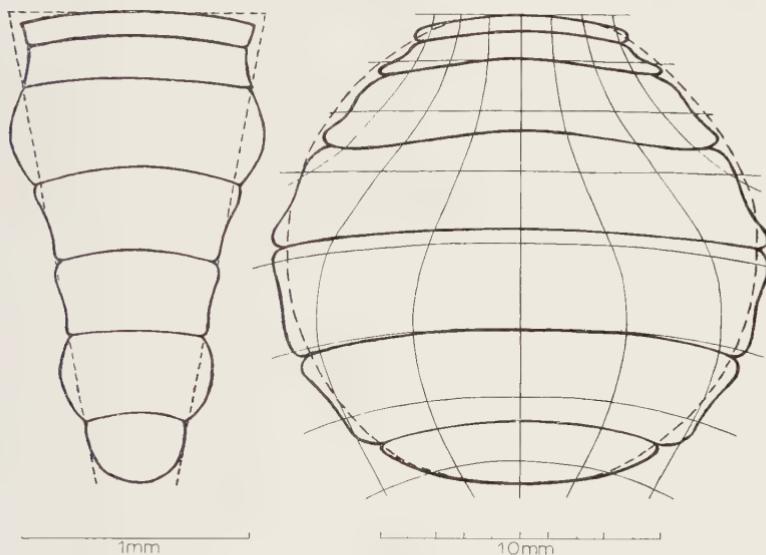


FIG. 4.3. TO ILLUSTRATE DIFFERENTIAL GROWTH AROUND A GROWTH CENTRE

Surface views of abdomina of young (left) and mature female pea crab, *Pinnotheres pisum*, reduced to the same total length. The broken lines show that growth changes a virtually triangular plate into a virtual disc. The thin lines superimposed on the mature abdomen show how a square grid superimposed on the young abdomen would be distorted by subsequent growth; they show how growth is graded from a centre of maximal rate in the mid line, near the junction of fifth and sixth abdominal segments.

(After Needham, 1950, modified)

However, the abdomen is a thin plate so that as it grows it can buckle outwards in the centre. Not only is this mechanically tolerable but it is probably a positive biological advantage since it enlarges the brood pouch below. Again, the lens of the vertebrate eye, to judge from the histological picture of the lens fibres (Fig. 4.4) appears to grow maximally near its centre. However, it also recruits new fibres round the equator, at the junction with the simple anterior epithelium; these elongate meridionally to fold round the lens. Since the lens is a more solid three-dimensional structure than the abdomen of *Pinnotheres* it seems that both components of growth must build up considerable pressure near the centre,

and this may explain the density gradient from the centre outwards, a very valuable property for minimizing the usual optical aberrations of lenses.

Another instance of differential growth with a useful basis, of a different kind, is that of the insect corpus allatum (Novak, 1954), which secretes the juvenile hormone (p. 381), preventing metamorphic change. The gland grows

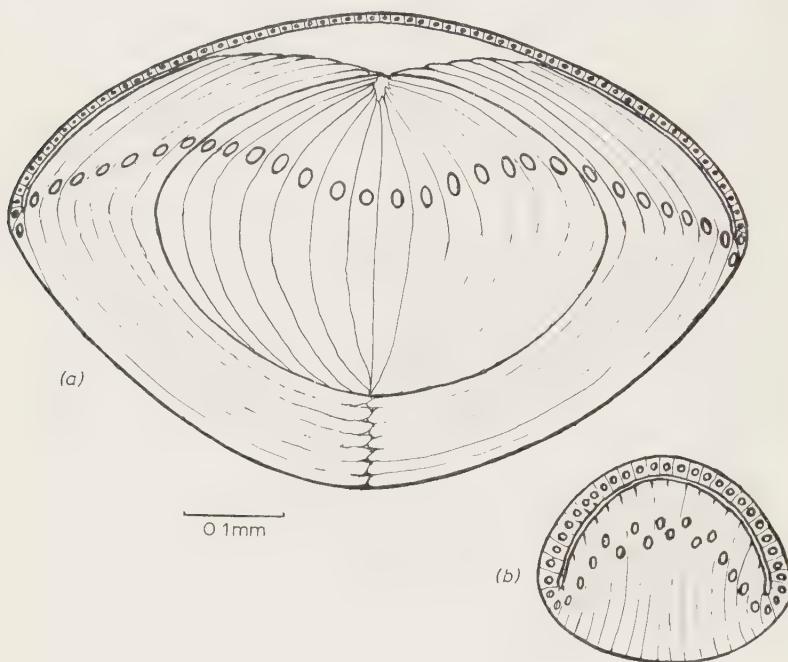


FIG. 4.4. DIAGRAMMATIC SECTIONS OF HUMAN LENS

(a) Meridional section of lens to show the form of the lens fibres and their recruitment from unspecialized lens epithelium, round the equator. These elongate and wrap round the lens nucleus (heavy outline). (b) Section of embryonic lens showing the initial focus of growth in the centre of the lens nucleus.

more slowly than the rest of the body, so that eventually the concentration of its hormone in the body falls below threshold and the insect metamorphoses at the next moult.

Other well known "functional" patterns include the disproportionate growth in diameter of the legs of large terrestrial animals. Since they must bear the weight of the whole body, ideally their cross sectional area should increase as the cube of the linear measurements of the body as a whole. It is found that the diameter does in fact increase as the  $3/2$  power of the other linear measurements, and on land they rapidly become too heavy to move at sizes much greater than that of the elephant. D'Arcy Thompson (1942) discussed

many other examples of this kind, where functional requirements appear to determine differential growth.

Instances of this kind conform to a simple mathematical relation of the form  $y = ax^b$  where  $a$  and  $b$  are constants. This is the relation of *simple allometry* (Huxley *et al.*, 1941), previously termed heterogony (Huxley, 1932); it has been the most fruitful of mathematical aids in the analysis of differential growth. Space does not allow a detailed consideration here but it may be noted that the relation is not restricted to the kind of functional situation just considered. Huxley (1932) favours a more general biosynthetic basis for it: it may be expected to apply whenever there is a constant ratio between the specific growth rates of the two measurements,  $(1/y) \cdot dy/dt / (1/x) \cdot dx/dt$ . Elimination of  $t$  and integration then gives the logarithmic form of the above relation:  $\log y = b \log x + \log a$ . This is the equation to a straight line, one of the technical virtues of the relation.

It has been found to fit also a number of phylogenetic trends in the measurements of adult animals. This has been termed simple *allomorphosis*, as distinct from the ontogenetic phenomenon, heterauxesis or *alloauxesis*. If  $b$  is greater than unity then  $y$  shows tachyauxesis or tachymorphosis relative to  $x$ , and if less than unity—bradyauxesis.

The relation cannot fit all measurements which might be compared: Haldane has shown that if it applies to  $x$  and  $y$  then it cannot hold for sub-units of  $x$  compared with  $y$ . Again, for reasons already given it seems theoretically improbable that the ratios between specific growth rates, in length, breadth and depth, say, are constant throughout the body. It is equally unlikely that the ratio for any pair of measurements is constant throughout the growth cycle, which is often asynchronous between organs (Fig. 4.1). In some cases even the simple linear relation  $y = ax + b$  seems adequate (Thompson, 1942): it represents strongly differential growth when  $b$  is large. In other instances (Williams and Needham, 1938; Needham, 1950a, 1957), however, a more complex relation is necessary. Nevertheless, the simple allometry relation is very useful and its potentialities, particularly for taxonomic purposes, have not yet been fully exploited (von Bertalanffy, 1960). In place of a single pair of measurements  $x, y$ , considered at some arbitrary moment of ontogenesis or of phylogenesis, the taxonomist can now express the relationship between the two measurements throughout the time span; there is no need to know the age itself, which often is not ascertainable. The relation has solved some interesting taxonomic problems (Reeve, 1940, 1941; R. Clarke, 1950). It has also helped with other problems (Needham, 1935) in addition to the many discussed by Huxley (1932).

In principle, allomorphosis could be a simple consequence of ontogenetic alloauxesis, for instance in cases where there is simple overstepping (De Beer, 1951), but more usually (Fig. 4.5) there is ontogenetic divergence during evolution and often the constants in the two relations between a particular pair of measurements,  $x, y$  are very different (Hersh, 1941). Biological significance has

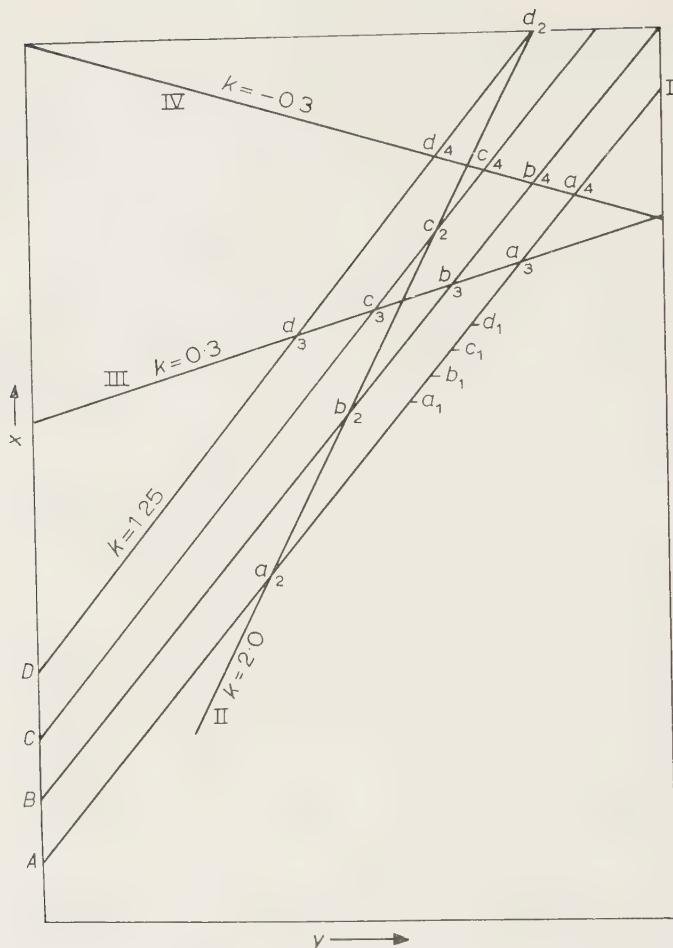


FIG. 4.5. GRAPHS ILLUSTRATING POSSIBLE RELATIONSHIPS BETWEEN THE HETEROAUXESIS OF TWO MEASUREMENTS  $x$  AND  $y$  IN FOUR SPECIES,  $A$ ,  $B$ ,  $C$ ,  $D$  AND THE ALLOMORPHOSIS OF THEIR VALUES  $a$ ,  $b$ ,  $c$ ,  $d$  IN THE ADULTS OF THE FOUR

Supposing that the heterauxesis graphs of all were coincident, on  $A$ , then the allomorphosis graph (I) also would be coincident. If they are parallel the allomorphosis graph may be steeper (II), less steep (III), or even have a negative slope (IV). In addition the graphs of heterauxesis might not be parallel.  $k$  is the exponent of the allometry relation.

been attributed to many instances of simple allomorphosis (Rensch, 1956), and the determining factors may be different from those which determine allauxesis between the same measurements.

In some instances it appears that differential growth itself may call the tune, rather than being determined by functional requirements. The number of neurons required in the brain, particularly in the association areas, does not

increase greatly with body size, if no great increase in intelligence is required, and therefore the organ could increase with extreme bradymorphosis during evolution and still meet general requirements (Rensch, 1956). In fact, however, there is only mild bradymorphosis (De Beer, 1940) in the various lines of vertebrates, and so the brain has provided, for neural evolution, far more material than can be immediately exploited. This may account for the vast silent areas of the human brain and the relatively small ill effects of the normal heavy, continuous loss of brain cells throughout adult life. Even among vertebrates with a much smaller brain size than the primates the larger types are the more intelligent—the rat than the mouse, and large races of hen than small (Rensch, l.c.). The phenomenon may be viewed from another angle: this may be the most important reason why increase in body size has been the most common of evolutionary trends, wherever counter-selection has not been too severe.

From the introductory considerations in this section it is to be anticipated that any simple practicable mathematical approach tends to oversimplify the actual growth pattern. Richards and Kavanagh (1945) have shown how complex and difficult to operate is a relation which aims at defining growth in detail throughout a two- or three-dimensional structure; it is complex enough even throughout a single dimension (Medawar, 1944; Hewlett, 1944; Needham, 1950). Unfortunately the known spatial patterns of growth are mostly complex, with a number of foci of maximal rate, and they vary considerably between animals. They are more amenable (Fig. 4.3) to simple geometrical (Thompson, 1942) than to simple algebraical treatment.

Another simple pictorial method of representation (Fig. 4.6) is based on the geographical contour map. Here the mean growth rates, as percentage increments, of the seven segments of the fifteen pairs of walking legs of the centipede, *Lithobius*, are plotted in order, evenly spaced on a grid, giving a picture of the spatial distribution of growth rates throughout the system of limbs. The contour lines help to visualize the pattern. It is seen that the pattern is relatively simple, with smooth gradients, indicating a high degree of integration throughout the system. This is the more remarkable because the limbs are interconnected only indirectly, via the trunk. However, they do have some kind of integrated representation in the nervous system, which has an important share in the control of growth (p. 352).

Minor irregularities, in the form of the ridges and troughs in the region of the eighth to twelfth limbs are due to the mode of initial development (Attems, 1930). In this region alternate limb rudiments are short and so must compensate by a differentially higher growth rate subsequently (Needham, 1945). The short limbs are in the body segments of the short terga typical of the lithobiomorphs. In some features, however, the growth patterns in the series of terga and sterna differ from that in the limb series. In addition, the patterns in the female differ in detail from those of the male.

The system of thoracic limbs of the isopod Crustacea (Needham, 1943)

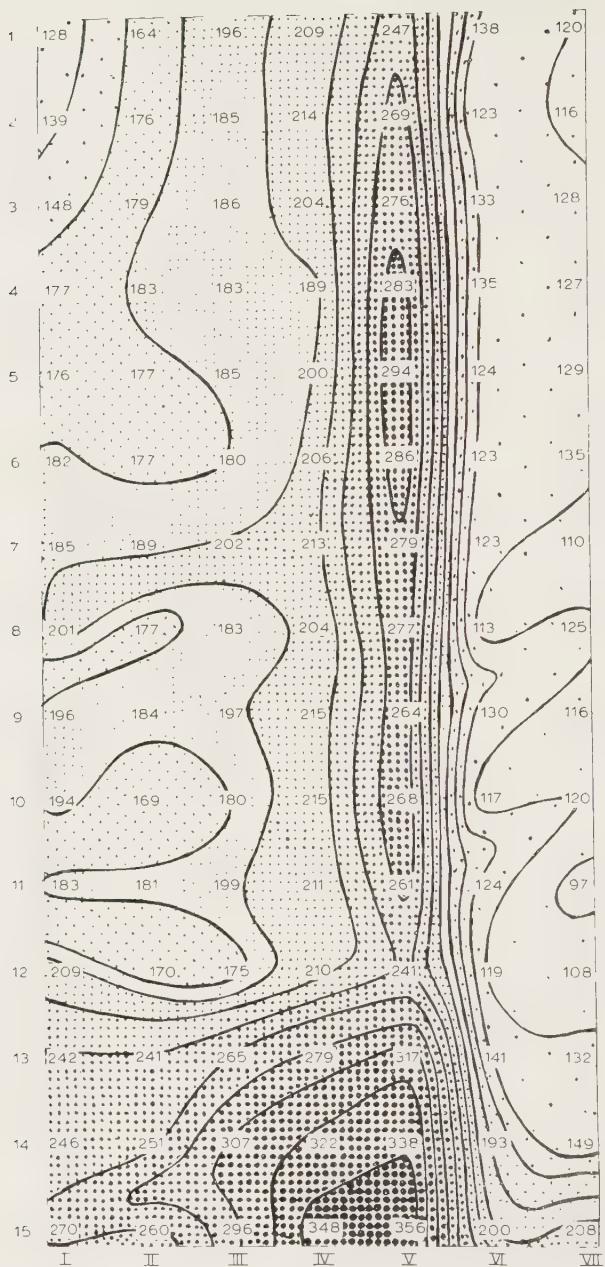


FIG. 4.6. GROWTH CONTOUR MAP FOR THE LENGTH OF THE SEVEN SEGMENTS (I-VII) OF THE FIFTEEN WALKING LEGS OF THE CENTIPEDE, *Lithobius forficatus* (L.)

The contour lines have been drawn round the values shown, which are percentage increments between juvenile and adult stages.

shows a pattern generally similar to that in *Lithobius*. A comparative study by this method might be profitable, particularly in cases such as these where it is not feasible to apply Thompson's Cartesian transformation method. Like the latter it represents very simply what would prove difficult algebraically. Many spatial patterns, however, can only be described more qualitatively at present and the main types of these will now be considered.

The main subdivision is between disseminated or intussusceptive growth and localized or zonal growth. Intussusceptive growth is seen in the multiplication of the cilia of ciliate Protozoa (Lwoff, 1949, 1950). New rows are inser-



FIG. 4.7. TO ILLUSTRATE GROWTH BY INTUSSUSCEPTION IN THE ANTENNULE OF *Ascellus meridianus*

Each broken line follows a particular intersegmental joint throughout the subsequent stages of growth. Intercalation is most rapid near the junction of flagellum and peduncle and follows a definite pattern.

ted and also new cilia within existing rows. The growth of the annulated type of antenna in insects (Imms, 1940) and in Crustacea (Fig. 4.7) approximates to this type, as shown by the intercalation of segments. However, the rate is maximal near the base of the flagellum, with a gradient distalwards, so that there is a tendency towards zonal growth. The stigmata or gill slits of tunicates (Fig. 4.8) provide another examples of this type of multiplication (Brien, 1948). This is an interesting example because it is the slits which multiply, so that new bars of tissue must grow across them and fuse, at each stage. Another example of orderly intussusception is that of the skin papillae of echinoid worms (Baltzer, 1934). Even more regular in pattern is the multiplication of mesenteries in a typical sea anemone (Fig. 4.9). Here new generations of mesenteries are regularly inserted, in pairs, in the centre of the exocoelic spaces between successive pairs of the mesenteries already present. The tentacles of scyphozoan and trachyline medusae are similarly recruited, and also those of tunicates,

whereas those of brachiopods, for instance, are added only at the end of the row. A regular but more complex intussusceptive pattern is found in the set of tentacles of some other medusae, and in the set of zooids of disconanth siphonophores (Komai, 1951). This resembles some of the more complex types of phyllotaxis in plants, and is better treated as a spatiotemporal pattern (p. 63).

In other instances, for example the insertion of new setae and new sensory plaques in the epidermis of the bug *Rhodnius* (Wigglesworth, 1945), there is a

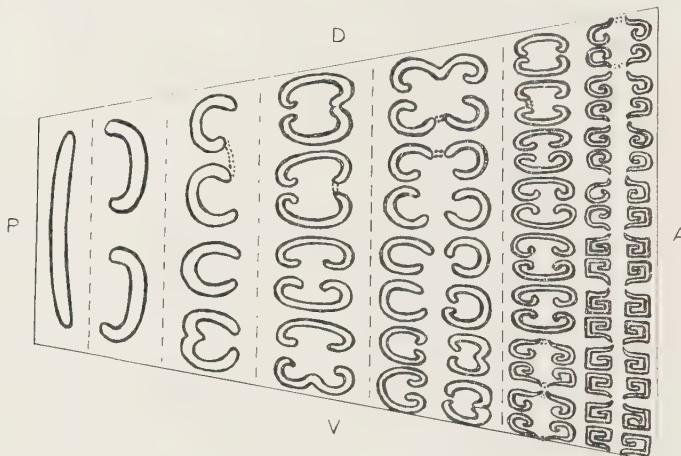


FIG. 4.8. DIAGRAM TO SHOW THE PATTERN OF GROWTH AND PROLIFERATION OF GILL-SLITS IN THE ASCIDIAN, *Corella*

The youngest slit, on the left, is set transversely to the posterior end of the pharynx wall. Further forward there are progressively older, more mature slits, formed by a regular pattern of subdivision, elongation, and curving. This depends on growth of the surrounding tissues, with periodic fusions across the centres of the slits; the dotted lines indicate regions of recent fusion. Subdivision proceeds about twice as fast in the dorso-ventral as in the antero-posterior axis. Within the field of one parent gill the process is most advanced ventrally and anteriorly.

(Based on Brien, 1948)

less geometrically regular mode of intercalation, though it is uniform statistically. The replacement of cells lost by wear is usually disseminated throughout each tissue, continuously or discontinuously; the gut mucosa is repaired by cells proliferated in the crypts which are scattered throughout the wall of the viscera. Massive organs such as the vertebrate liver may grow by fairly uniform intussusception but the precise mode is far from certain (Abercrombie, 1957): the pattern is not easily elucidated in such amorphous organs. Cell division sometimes appears to be uniformly scattered throughout a growing organ or body, but differential rates are more usual, even in the early embryo (J. Needham, 1942). In most cleaving eggs proliferation is already faster at the animal than at the vegetative pole, and during subsequent development the differential

pattern becomes progressively more complex (p. 37). The general conclusion is that at all stages growth is more by means of localized growing points or zones than by uniform intussusception. Zones may be superseded by others,

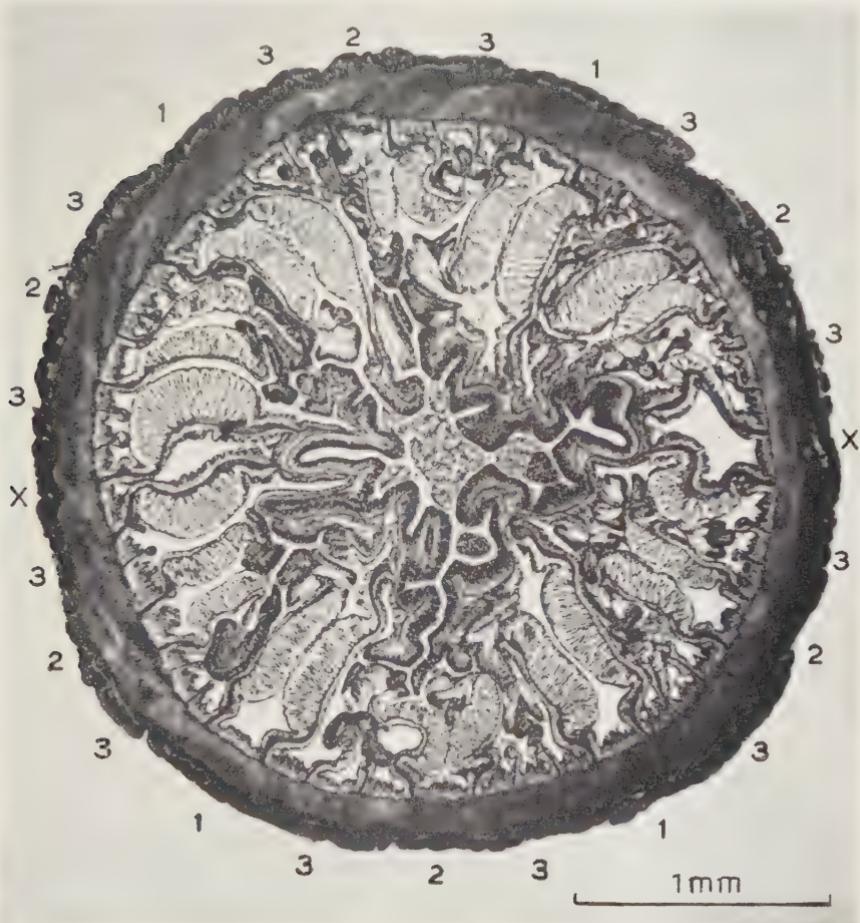


FIG. 4.9. THE PATTERN OF PROLIFERATION OF MESENTERIES IN A TYPICAL SEA ANEMONE, AS SEEN IN TRANSVERSE SECTION OF *Cereus*, NEAR THE BASE OF THE STOMODEUM

The successive cycles of paired mesenteries are numbered in order. X, X are the two pairs of directive mesenteries of the first cycle, which alone have muscle banners not facing each other across the endocoel.

(Photo: J. S. Haywood)

and some move from one region to another in the course of time, quite apart from the automatic shift caused by the piling up of the products of their own activity. In this last situation they are conventionally regarded as remaining in the same place, e.g. at the ends of twigs, but if the zones genuinely shift with time then we have a spatiotemporal pattern (p. 62).

The number of possible spatial patterns based on localized growth zones is strictly limited if we accept the mechanical sanction that new cells can be placed only where there is space for them, usually at surfaces. Growth zones then must be at the most two-dimensional or laminar, and they may be one-dimensional, in the form of a line or strip, or of a closed ring. In the extreme case there may be a mere growth point. By unimpeded proliferation, while maintaining its punctate form, such a zone can produce only linear filamentous

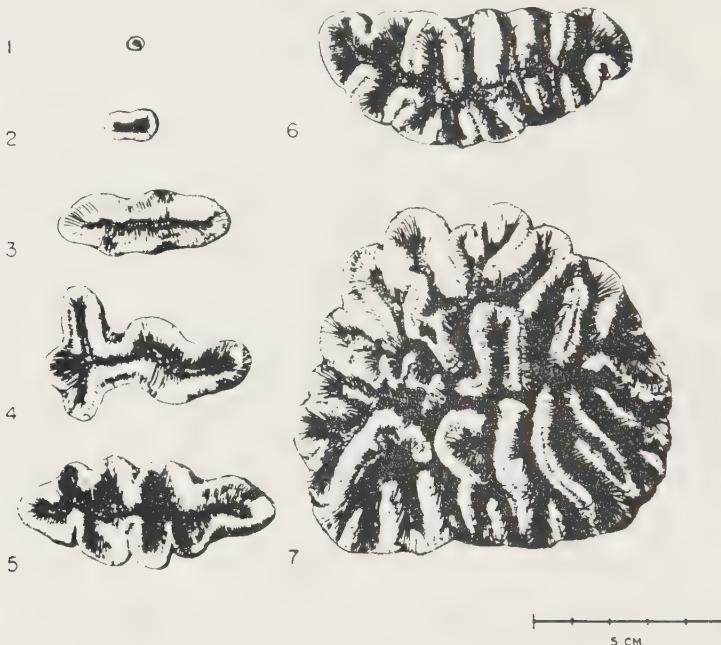


FIG. 4.10. PATTERN OF GROWTH OF THE SKELETON OF THE CORAL *Manicina areolata* SO AS TO ACCOMMODATE THE GROWTH OF THE POLYPS BY ELONGATION OF THE ORAL DISC AND "INTRATENTACULAR BUDDING" OF NEW STOMODAEA

(From T. F. and N. I. Goreau, 1961, Biol. Bull., Wood's Hole, 118.)

structures, though these may coil into spirals, as in some of the Foraminifera (Fig. 9.1) and may branch and form other secondary shapes.

A zone of the second grade, in the form of an open line or band, can add material throughout its length, and in that direction, forming a filament as in the previous case. Alternatively, it can proliferate at right angles to its length, forming a rectangular structure, or both, forming a trapezium. The filamentous algae provide examples of the first type and the thyroid, salivary and gastric and other glands also at first grow as solid cords. A line of this kind can branch, and the meandrine corals (Fig. 4.10) illustrate maximal exploitation of such a mode of growth, in their elongating oral disc and skeleton, so as to occupy the whole available surface area as effectively as by a true two-dimensional mode. The

growth of the human cerebral hemispheres, which the meandrine skeleton so much resembles superficially, in fact is a device to accommodate maximal area of surface in minimal cranial volume, differing from the problem of the coral, therefore, by one dimension.

The mesenteries of sea anemones (Fig. 4.9), considered as entities, are examples

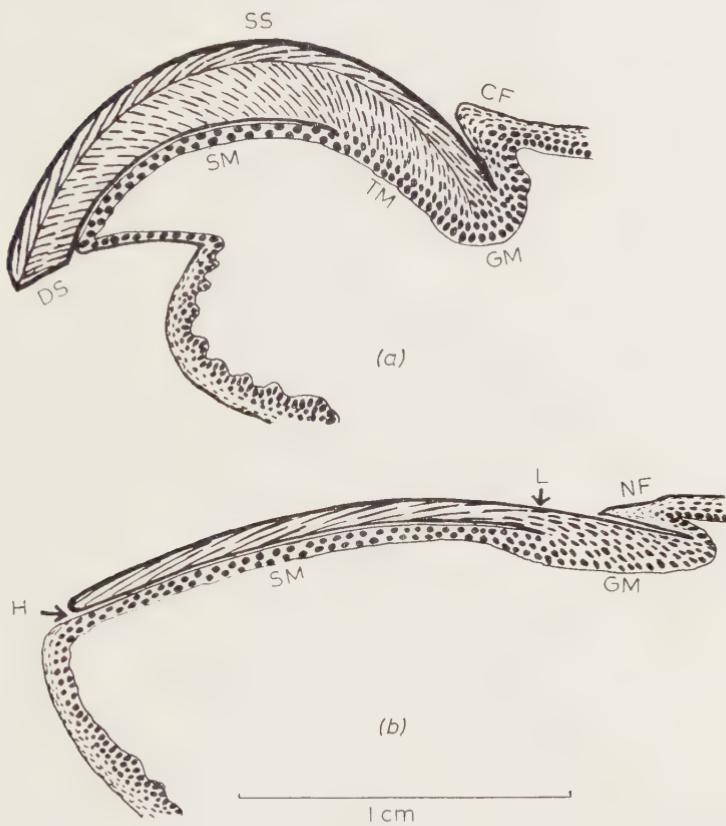


FIG. 4.11. DIAGRAMS OF SAGITTAL SECTIONS OF: (a) THE CLAW OF ONE OF THE CARNIVORA AND (b) THE FINGER NAIL OF MAN

*CF*, claw fold; *DS* deep stratum; *GM*, germinal matrix; *H*, hyponychium; *L*, anterior edge of lunule; *NF*, nail fold; *SM*, sterile matrix; *SS*, superficial stratum; *TM*, terminal matrix.

(Based mainly on Le Gros Clark, 1958.)

of approximately rectangular structures produced by a vertical growth strip in the column wall. In the common anemones, as already noted (p. 39) new mesentery-forming strips are intercalated at even intervals round the column but in the Cerianthidea and Zoanthidea proliferation is restricted to one or two permanent growth strips either in the ventral endocoel or on either side of it.

This linear type of growth zone also could produce spirally coiled filaments

or spirally rolled plates, and it could also lead to other asymmetrical forms. The human nail (Fig. 4.11) approximates to an arched plate produced by a relatively narrow band of cells underlying the lunule region. Strictly speaking it is only an approximation to such a zone, although the result is such a good approximation to a plate, growing continuously at one edge. In the first place the germinal region, if we include that hidden by the nail fold, is nearer to a half disc than a mere line, and in the second place the flat nail has probably evolved from a domed scale, as seen on the digits of reptiles, by the suppression of the anterior half of the germinal matrix, and by other modifications. In the claws of other mammals this anterior half of the matrix still produces columns of keratinized cells, abutting at an angle to those of the upper layer (Fig. 4.11). The claw is also strongly arched from side to side so that it illustrates the evolutionary complication of a growth pattern as admirably as the nail shows simplification. The hoof of perissodactyls provides another interesting modification of this structure.

A ring-shaped zone can grow simply as a ring, in the circumferential direction only, or it can grow both in this and in the radial direction, forming a disc or plate, as in the scales of teleost fish (Fig. 3.1). The plates of the corona of echinoids (Fig. 6.8) grow mainly round the edge, while the bones of the vault of the mammalian skull have a much more tortuous circumscribing growth zone. A ring may also grow in a direction perpendicular to its plane forming a cylinder.

Coelenterates grow in length in this way from growth rings, and in the colonial hydroids there is a ring near the tip of each branch. Like plants the hydroids show both monopodial and sympodial modes of budding (Figs. 4.12, 4.13, 4.14), the former characteristic of the Gymnoblastea and the latter of the Calyptoblastea. In the monopodial type (Fig. 4.12) the main growing point lies just below the oldest zooid or hydranth, which tops the colony, while secondary growth zones have a similar position on each branch, the longest and oldest of which are at the base of the colony. Each growth zone elongates its own region of the stem, or hydrocaulus, and periodically buds off laterally a new hydranth together with its own growth zone, potentially a new branch.

In the sympodial method the growth zones are similarly located, at the bases of the terminal hydranths of the moment, but each zone ceases to grow soon after that hydranth is formed. Consequently further growth depends on the zones of succeeding generations of hydranths, each of which overtops its parent before the growth activity passes in turn to the next hydranth on that branch. The oldest hydranth therefore is at the base and not at the tip of each stem and branch. When successive daughter hydranths are formed in order on opposite sides of the branch a zig-zag type is formed, as in *Obelia* species (Fig. 4.13).

The higher calyptoblasts have developed a "pseudomonopodial" habit (Fig. 4.14), simply by bending the stalk of each newly formed hydranth so that the bud of the next, together with its growth zone, continues the line of the

main axis of that stem or branch, and appears to terminate it. In contrast to the true monopodial type, therefore, a growing point, and not the oldest hydranth, tops each branch. The budding may run ahead of hydranth development



FIG. 4.12. APEX OF MAIN STEM (HYDROCAULUS) OF THE GYMNOBLAST HYDROID, *Bougainvillia*, TO SHOW THE MONOPODIAL MODE OF GROWTH OF NEW HYDRANTHS

The largest, oldest hydranth is at the apex, and the youngest is immediately below this (and seen as a dark mass in line with the main stem). Hydranths increase in size in order, towards the base. Their relative ages are shown also by the number of daughter hydranths on their own branches; these grow and proliferate in the same monopodial way.

(Slide: N. M. Needham; photo: J. S. Haywood)

(Fig. 4.14). In this type the successive hydranths are usually on the same side of the branch. In some cases, however, a pair of hydranths is formed at a time, on opposite sides of the branch, and the growing point then appears even more

clearly terminal, with a pair of young hydranths symmetrically placed on either side of it.

The pennatulid coelenterates show ideal monopodial budding, to such an



FIG. 4.13. APEX OF THE HYDROCAULUS OF THE PRIMITIVE CALYPTOBLAST HYDROID, *Obelia*, TO SHOW THE SYMPODIAL MODE OF PROLIFERATION AND GROWTH  
The youngest hydranth, with tentacles not yet formed, has already overtopped the next older individual below it and a cushion-like bud on its neck will soon develop into the next hydranth, and repeat the process. Note the alternate arrangement of successive buds.

(Photo: J. S. Haywood)

extent that the whole colony is often regarded as a system of buds on the sides of a single large parent zooid, but this view is a matter of personal taste. A rather interesting parallel is the system of tube-feet or podia of the crinoid echinoderm, *Antedon*; these are outgrowths of a radial water-vessel which appears to be simply a gigantic elongation of the first formed podium. The

solitary hydroid, *Hydra* grows in essentially monopodial fashion, from a growth ring just below the crown of tentacles (more distal than in colonial types), but the new material does little more than keep pace with a resorption of old



FIG. 4.14. APEX OF A COLONY OF THE CALYPTOBLAST, *Plumularia*, TO ILLUSTRATE THE PSEUDOMONOPODIAL MODE OF BUDDING, WHICH HAS EVOLVED FROM THE SYMPODIAL METHOD

(Photo: J. S. Haywood)

material at the base of the pedicel (Brien, 1953). This is therefore a remarkable case of "turnover" (p. 187) at the cellular level. In fact growth shows a more positive balance than this, and a regular sequence of buds also are formed and released. In experimentally isolated pieces of the stem of *Obelia* (Berrill, 1949a) the phenomenon of cellular turnover has an even more dramatic manifestation,

since the piece actively migrates by laying down new cells at one end and resorbing cells at the other. At the intracellular level this has something in common with the mode of movement of *Amoeba*, and of the spirally shaped organism described by Picken (1940), and it is an intriguing possibility (cf. p. 5) that amoeboid movement also has something in common with processes of growth and resorption. Growth as an aid to movement is less common in animals than in plants—and less common than movements as an aid to growth.

The gut of the insect imago grows in the same general way (Fig. 6.5) from growth rings (Henson, 1946), and it is the usual mode for many glands and other tubular structures. The heart of the tunicate, *Ciona*, grows from rings at either end, and there is resorption of effete muscle cells in the middle (Millar, 1953). By repeated branching some tubular organs become massive; examples are the young vertebrate liver, the kidney and many glands. The mature liver (p. 40) possibly continues to grow in this way. The pro- and mesonephroi of vertebrates develop as a number of separate tubules which grow back to join, or to form, the collecting duct. By contrast, the metanephros of the mammals develops in precisely converse order: the ureter is formed first, growing forward from the cloaca, and branches repeatedly at its anterior end to form the kidney tubules. This illustrates the plasticity of growth processes under evolutionary forces, and necessitates caution in using growth patterns as taxonomic and phylogenetic evidence.

Feathers (Fig. 4.15) are effectively cylinders produced by a growth ring at the base of the bud, which is initially a hollow cone. One of the interesting features of feather growth is that the distal part of the epidermal cylinder produced eventually splits along one side, designated ventral, and opens out to form an almost flat plate, the mature vane (Fig. 4.15 (a)). In addition a series of parallel splits occur along lines of weakness which make an angle with the main dorsal thickening, or rachis, resulting in a series of "barbs" on either side (Fig. 4.15 (b)). These are so formed as to possess an ingenious set of barbules to anchor them loosely to the neighbouring barbs, so forming a structure unrivalled for its lightness and air-insulating properties, combined with considerable mechanical strength and other useful properties. The base of the cylinder persists unfrayed, as the calamus or quill.

Isochrones, that is lines of material laid down at the same time are not, as might be expected, parallel to the barbs but cross them at an angle (Fig. 4.15 (a)) so that while the equivalent of one complete barb is being formed at any one moment, on each side of the rachis, this consists of sections of a large number of different barbs. An analogy at the molecular level is seen (Fig. 14.2) in Dalgleish's theory of protein synthesis (p. 202). The problem of the way in which these complex barbs are formed is therefore even greater than appears at first sight. Pigment is often laid down discontinuously in the growing feather (Fig. 4.15 (a)) and the bars of colour then neatly plot an isochrone. On the opened vane this makes a small angle with the transverse axis, as would be expected but, as shown, the angle is not always distal to that axis, at its

junction with the rachis; presumably either deposition is not always simultaneous throughout the germinal ring or this bears a somewhat variable angle to the transverse plane of the cylinder.

Feathers are often rather asymmetrical (Fig. 4.15 (b)), depending on their



FIG. 4.15 (a). TWO FEATHERS, TO SHOW RELATIONSHIPS BETWEEN THE ALMOST TRANSVERSE BARS OF PIGMENT AND THE OBLIQUE BARBS

Slight deviations from a perfectly transverse orientation of the pigment-bars may result when the cylindrical bud opens out to form the flat vane, though the deviation is in opposite directions in the two species illustrated. There is asynchrony between the two lateral halves of each bar, especially towards the base of the feather.

(Selected by J. Hull; photo: J. S. Haywood)

position in the plumage. The difference in threshold of sensitivity to growth factors of the two sides may be so great that in lateral gynandromorphs the individual feathers near the mid-line are gynandromorphic in colouring and texture. Other aspects of this particularly fascinating piece of morphogenesis are described by 'Espinasse (1939) and by Lillie and his pupils (Lillie, 1942).

When a growth ring grows circumferentially as well as perpendicularly to its plane then conical structures are formed. The feather in fact begins as a cone, while the shells of gastropods and of some cephalopods are typical cones which usually also become coiled, through unequal growth in the direction perpen-



FIG. 4.15 (b). DIAGRAM OF A PORTION OF THE CYLINDRICAL BUD OF A FEATHER

The upper end has been sliced obliquely to show the rachis down the back, and the way in which the rest of the cylinder splits into barbs. Near the base of the portion is an isochrone of pigment, deposited approximately parallel to the base of the cylinder and passing across a number of barbs.

(Based mainly on 'Espinasse, 1939)

dicular to the plane of the generating ring. If growth in this direction is symmetrically graded, from a minimum at one point to a maximum at the opposite end of the same diameter of the ring, then a plane spiral is formed. If the two are not diametrically opposite then a helical coil results (Fig. 5.2). This is seen also in the horns of bovid artiodactyls, in the tusks of pigs, and in other structures. Very shallow cones, asymmetrical in one or both longitudinal planes,

are found in the shells of brachiopods, lamellibranchs, ostracods and Conchostraca. By further flattening the cone passes into a flat plate, as in one valve of *Pecten* and some other lamellibranchs, and so converges with such structures as the teleost scale (p. 44).

Teeth with single roots and simple crowns approximate to a cone (Fig. 4.16)

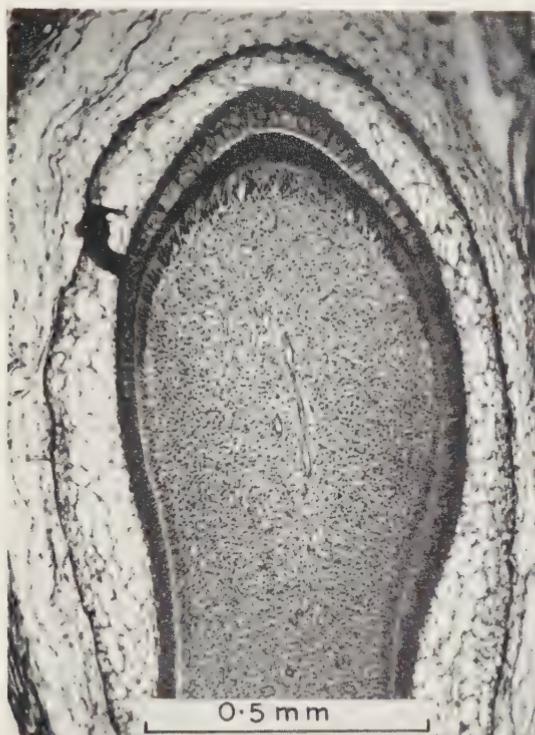


FIG. 4.16. VERTICAL SECTION OF DEVELOPING TOOTH OF A MAMMAL

In the centre is the dermal pulp-cavity, surrounded by a dermal epithelial layer of odontoblasts, secreting dentine externally (the black cap). External to this is the layer of ganoblasts or ameloblasts which secrete enamel on their inner faces (the white cap). The ganoblast layer is the inner wall of an epidermal "dental lamina," the outer wall of which appears as a dark line, limiting the spongy tissue filling the cavity of the lamina. The slide is interesting histologically, therefore, in showing a columnar dermal epithelium and an epidermal connective tissue.

(Slide: Robert Watt; photo: P. L. Small)

formed by the very nicely coordinated activities of a layer of epidermal ganoblasts or ameloblasts on the outside and one of dermal odontoblasts on the inside. At first these cover the whole of the slant surfaces, but later are effectively restricted to a ring at the base, at least in those teeth which grow persistently. They are usually slightly, if not more, asymmetrical, and therefore the tusk of the narwhal is outstanding as much for its resultant straightness as for

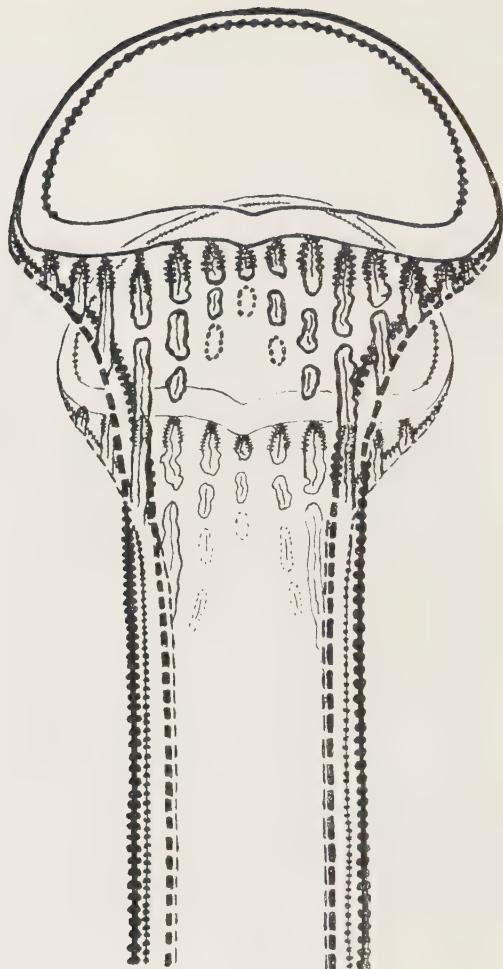


FIG. 4.17. DIAGRAM OF A LONGITUDINAL SECTION OF THE HEAD AND PART OF THE SHAFT OF A MAMMALIAN LONG BONE AT TWO STAGES OF ITS GROWTH, TO ILLUSTRATE GROWTH IN LENGTH AND WIDTH, TOGETHER WITH THE MODE OF REMODELLING OF HEAD INTO SHAFT

The lighter lines are those of the earlier stage. Bone is being deposited on all beaded surfaces and removed from all broken surfaces. The shaft is becoming wider and its wall thicker. Growth in length begins in the trabeculae bracing the cavity of the metaphyseal region. The head grows in width by recruiting more trabeculae peripherally, those in the centre being direct prolongations of those of the earlier stage. The wall of the head region is remodelled into shaft by erosion at the outer face and deposition on the inner face. At any point, therefore, material might be deposited, and later removed: finally new material might be laid down there, as the diaphysis widens. The epiphysis grows in proportion, from a growth zone below the articular cartilage. Trabeculae are progressively removed from the centre of the diaphysis, to enlarge the marrow-cavity.

(Based mainly on Leblond)

its great length and perfect conical form. It probably owes its straightness to the rifling, which is evident as spiral ridges and grooves on its outer surface, and which is thought to be due in turn to a progressive rotation in its socket (Thompson, 1942, p. 910). This neutralizes all asymmetry in growth rate



FIG. 4.18. ANTERIOR END OF THE TAPEWORM, *Dipylidium*, TO SHOW THE LINEAR ORDER OF PROLIFERATION OF PROGLOTTIDS FROM A BUDDING ZONE IN THE "NECK" REGION, AND THE SUBSEQUENT GROWTH IN LINEAR ORDER

(Photo: J. S. Haywood)

round the germinal ring. D'Arcy Thompson suggested that the progressive twist is due to the asymmetrical torque of the animal's locomotor mechanism, the tusk acting as a relatively fixed fulcrum for this. If this is the correct explanation then the pitch of the rifling should decrease as the tooth grows larger and heavier, and the torque more powerful; the pitch does, in fact, decrease progressively towards the base. The individual dental lamellae are not twisted so

that the rotation certainly occurs at each point after the dentine has been deposited, and the tooth is not so rigidly fixed in its socket as we subjectively imagine!

A growth zone of the third or laminar grade can produce a variety of forms; the most common is a subterminal plate which produces a solid cylinder, or a prism, or some other shape. An example is in the head of the mammalian long



FIG. 4.19. POSTERIOR END OF YOUNG POLYCHAETE WORM SHOWING PROLIFERATION AND GROWTH OF SEGMENTS FROM A POSTERIOR GROWTH ZONE, JUST IN FRONT OF THE PYGIDIUM

(Photo: J. S. Haywood)

bones (Fig. 4.17); the shaft is a hollow cylinder but it begins as a solid terminally, the central resorption occurring later. Other examples are the budding zone in the neck region of cestode worms (Fig. 4.18), the penultimate growth zone of annelids (Fig. 4.19) and the subterminal zone in the arms of starfishes.

It is interesting that the zone is usually subterminal and in some cases there are evident reasons why it is not absolutely terminal. The bearing surfaces of mammalian long bones must be rigid and functional from an early stage and so the growth zone lies between the terminal cap or *epiphysis* and the shaft or *diaphysis*, the main part of the shaft. Similarly the scolex of the cestodes must

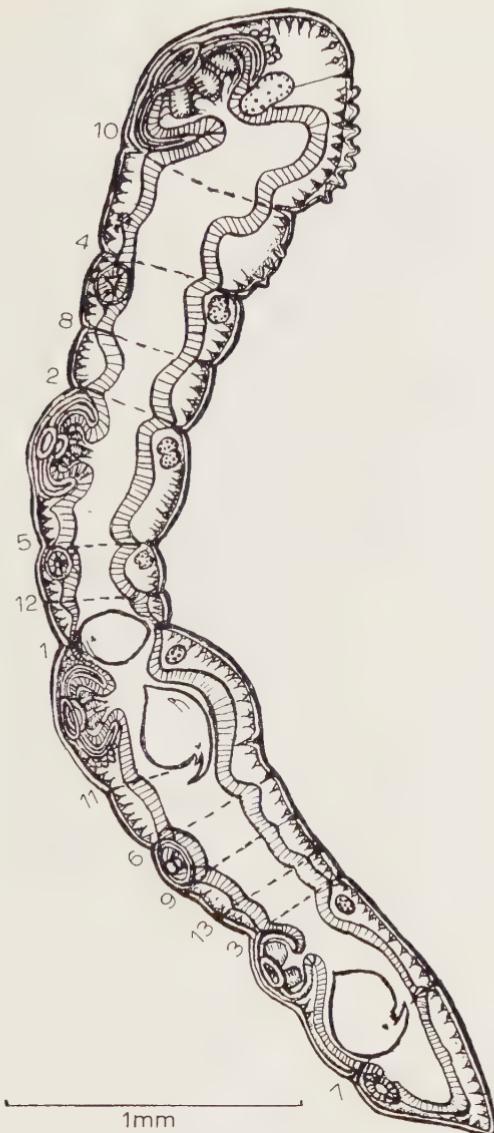


FIG. 4.20. DRAWING OF *Microstomum lineare* (Rhabdocoelida) TO SHOW THE SEQUENCE OF FISSION PLANES ASSOCIATED WITH ITS NEOTOMIC METHOD OF ASEXUAL BUDDING  
The pharynx and cerebral ganglia are visible in the more mature buds.  
The common gut contains several ostracods.

be fully developed at the outset. The most basal segments of the arthropod antenna contain the main muscles which actuate the organ, and so the growth zone is situated just distal to this (Fig. 4.7). In the pennatulids, again, the growth zone lies between the rachis and the calamus which must be used for

burrowing. Terminal zones occur in protected structures such as teeth, hairs and feathers, but in those which are unprotected the growth zone is usually subterminal so that the tip provides protection, as in the roots of plants. Even

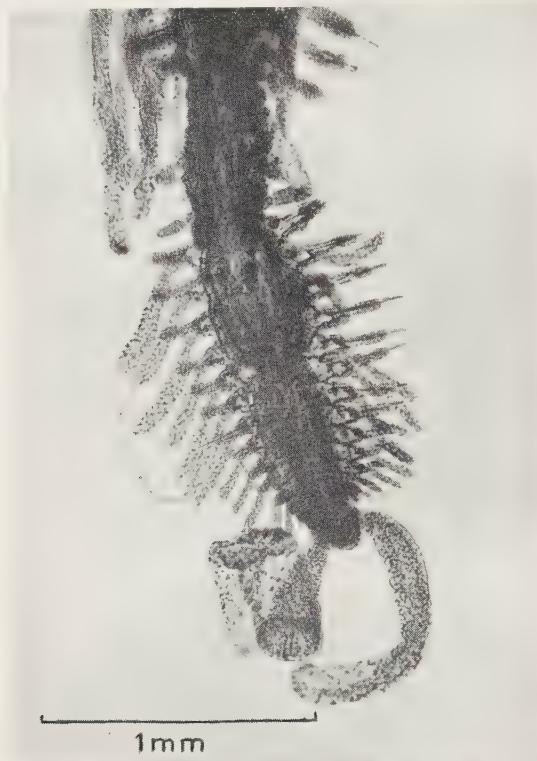


FIG. 4.21. PHOTOMICROGRAPH OF THE POSTERIOR END OF THE POLYCHAETE WORM, *Myrianida pinnigera*, TO SHOW THE METHOD OF ASEXUAL BuddING IN LINEAR ORDER FORWARDS

The posterior growth zone of the parent repeatedly divides transversely, the posterior portion becoming the posterior growth zone of successive buds. The two oldest, most posterior buds are clearly distinct as well as the large posterior pair of parapodia of the next two buds. Three parental segments are seen at the top.

(Photo: J. S. Haywood)

when the zone is terminal the proliferation rate may reach its maximum some distance along.

In the asexual budding of rhabdocoel (Fig. 4.20) and polychaete worms (Fig. 4.21), the transverse budding zone may be remote from the end of the body, however, and in rhabdocoels there may be many such zones arising in turn, in a regular pattern, approximately across the middle of existing individuals. As in the simpler case of the filament growing throughout its length (p. 42) there is no mechanical bar to this kind of growth provided the new

products are inserted uniformly in the direction of the long axis. In some polychaetes, however, there is a much more makeshift device (Fig. 4.22), the buds being placed laterally in an untidy bunch: in *Syllis ramosa* a profuse branching is associated with asexual reproduction (Berrill, 1952).



FIG. 4.22. POSTERIOR END OF THE POLYCHAETE, *Syllis ramosa*, SHOWING THE LUXURANT PROLIFERATION OF DAUGHTER WORMS AT MANY POINTS ON THE SIDE OF THE PARENT

Each daughter has the usual posterior growing point and remains attached to the parent by its anterior end.

(Photo: J. S. Haywood)

Initially, at least, the growth zone of feathers and teeth is a lamina in the form of a conical surface and that of a hair remains such a conical lamina throughout. It is a deeply sunken portion of the Malpighian layer of the epidermis (Fig. 4.23), and each cell of the lamina proliferates a column of keratinized cells on the outer side of the cone. The enamel produced by the cone of epidermal ganoblasts of the tooth germ is, by contrast, an extracellular secretion and almost

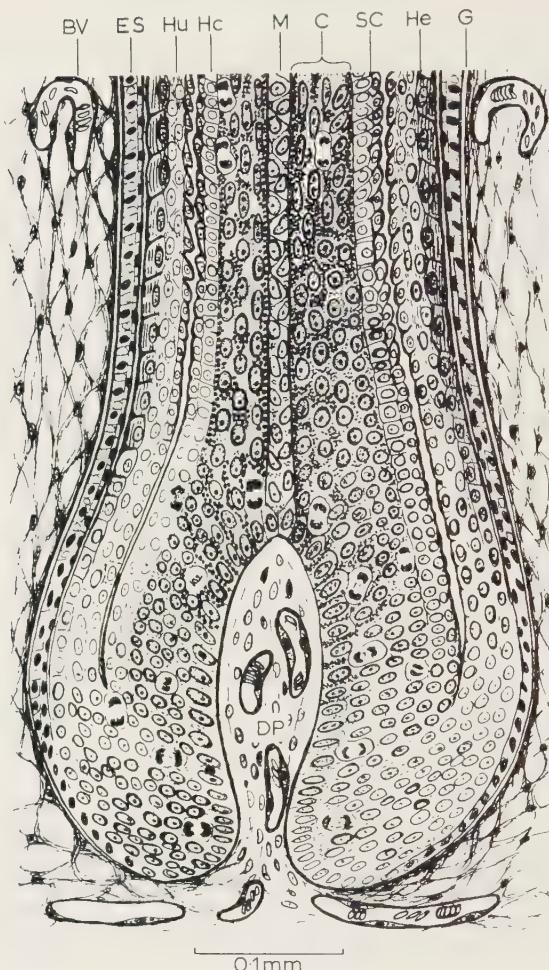


FIG. 4.23. DIAGRAM OF A VERTICAL SECTION OF THE BASE OF A HAIR, IN ITS FOLLICLE (ES), TO SHOW HOW THE CONCENTRIC LAYERS OF THE HAIR ARE FORMED FROM THE MALPIGHIAN STRATUM OF THE HAIR PAPILLA

The orientation of the mitotic figures indicates that each layer comes from a particular ring of cells in that stratum. The cleft between the inner sheath cuticle and Huxley's layer separates the protrusible part of the hair from the layers which remain lining the follicle. Some of the medullary cells and their progenitors in the Malpighian layer contain pigment granules. BV, blood capillary in the dermis; C, cortex; DP, dermal papilla; ES, external root sheath (epidermis of wall of follicle); G, glassy membrane (basement membrane); HC, hair cuticle; He, Henle's layer; Hu, Huxley's layer; M, medulla of hair; SC, inner sheath cuticle.

pure mineral (p. 162). The growth of hair presents much of interest (Montagna and Ellis, 1958): for the present purpose the main point is that each cell of the conical germinal matrix buds off cells in a continuous column, always of a

particular type, contributing to one of a number of concentric cylinders of cells which make up the solid hair (Fig. 4.23). Cells in the sides of the cone proliferate in a direction at first almost normal to its surface but later the mitoses which occur in the column of its progeny cells are orientated along the axis of the hair (Bullough and Lawrence, 1958). There must be either a very well integrated differential rate of proliferation between apex and base of the cone, or between the progeny of the various cells, or there must be extensive plastic movements between the progeny, to permit this. In support of the last explanation it is also noteworthy that the diameter of the mature hair is less than that of the bulb itself. Similar processes must occur in the finger nail (Fig. 4.11) since the columns lie eventually parallel to the surface of the digit although they grow out from the matrix initially as vertical columns; by contrast, those of the anterior part of the matrix of the claw remain as they begin, almost normal to the surface. It is believed that hairs are not homologous with reptilian scales, as nails and claws are, but it is noteworthy that the matrix of both is essentially a cone. During their relatively short evolutionary career hairs have developed a number of modifications, including the spines of the hedgehog and the porcupine, and probably the whalebone of the *Mystacoceti*. The horn of the rhinoceros may be a fused bunch of hairs. True hairs themselves vary greatly in length, mode of growth and other details.

Antlers may be regarded as solid products from the inner face of the conical germinal lamina of the velvet. This becomes branched and develops various asymmetries, in detail, which determine the tines. The antler grows only on its surface, by the activity of the overlying velvet, and therefore this becomes a particularly large and complexly shaped growth lamina. Other large laminae are seen on the surface of the bones of the cranial vault and of the plates of the echinoid corona, both of which grow on the surface as well as at their edges (p. 44). Bone is laid down on the outer side of the cranial tables but on the inner face of the echinoid plates (Deutler, 1926). The relation of this to the enlargement of the cavity within is considered later (p. 79).

The periosteum of the diaphysis of the mammalian long bones is a cylindrical growth lamina (Fig. 4.17), while the perichondrium of a typical cartilage can be a completely closed surface. The most extensive growth lamina is the Malpighian layer of the vertebrate skin, including the linings of fore and hind guts (Fig. 4.24). It is interesting also because proliferation is on the external face and functions merely to take the wear on the body's surface, this is the classical example of physiological regeneration (p. 5).

A spatial pattern which has always excited interest is the stripe patterns of the pelage of the zebra (Fig. 4.25) and tiger. The stripes of some animals are due to spatiotemporal patterns and will be considered in the next chapter but the zebra and tiger stripes are believed to develop simultaneously throughout the body: Henke (1933, 1935) refers to them as a simultaneous rhythm of standing waves. They are determined once for all during early ontogenesis, and give the impression (Fig. 4.25) that the determination in some way radiates

out in waves from well marked anatomical points. The main focus is in the centre of the forehead and from here the stripes of the head, trunk, tail and perhaps ears, seem to emanate as a single system—but greatly disturbed by the intrusion of the other systems radiating from five other foci, namely the dark areas of the muzzle and the pasterns. The pattern varies in detail in the different species of zebra (Thompson, 1942, p. 1091).

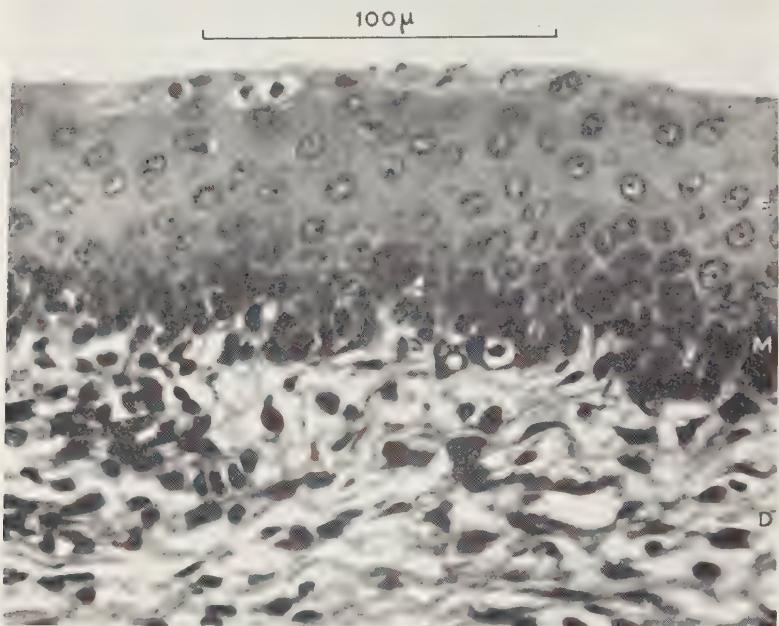


Fig. 4.24. VERTICAL SECTION THROUGH THE LINING EPITHELIUM OF THE OESOPHAGUS OF A MAMMAL TO SHOW PROLIFERATION OF EPIDERMIS, E, FROM ITS BASAL MALPIGHIAN LAYER, M

The cells become flattened towards the free surface, where they are scarfing off. The flattening is less marked than in the skin and the Malpighian and other layers are less clearly delimited. D, dermis.

(Photo: J. S. Haywood)

The pattern is very reminiscent of Liesegang's rings, produced by chemical reaction under special conditions (Hedges, 1932), but it is generally thought that anatomical stripes cannot be explained so simply. Nevertheless the intervening faint bands seen in some regions (Fig. 4.25) are very characteristic of Liesegang's ring formations. Perhaps the greatest difficulty in accepting the Liesegang phenomenon as the basis is that the tiger's stripes are replaced by spots or by roundels in some of the other large cats. Henke (1947) has discussed the basis of this kind of variation in another subject, the colour patterns of butterfly wings.

Apart from unusual cases of this kind it seems that the growth of most metazoan organisms and organs is due to the proliferation of relatively simple zones of cells in the form of filaments, rings and laminae. These proliferate cells in a constant direction, usually on one side of a filament or a ring and on one face of a lamina; there is sometimes growth along the line of the former and in the plane of the latter. In most cases cells are disposed so that growth does not cause tensions or distortions in the tissues; in exceptional cases where distortion does occur the results seem to have a special biological value.



FIG. 4.25. BURCHELL'S ZEBRA, TO SHOW THE PATTERN OF STRIPES IN THE  
PELAGE (*see text*)

The pattern differs in detail in other species.

Mechanical factors demand that growth shall be largely the work of localized growth zones, and much less due to cellular intussusception, but on the other hand biological and physiological requirements often necessitate that the gross growth rate in different directions shall conform to some simple relation, such as that of allometry, just as though growth were by disseminated intussusception. These simple relations, therefore, may often define the physiological requirement rather than the precise mechanism of growth. The actual pattern of growth during a particular time interval can usually be demonstrated by geometrical methods while in principle at least algebraical functions could define it as a continuous function of time and space.

Intussusception of an orderly kind is fairly common at the organ level. Both this and zonal growth can produce regular patterns of structure and of pigmentation.

## CHAPTER 5

*Spatiotemporal Patterns of Growth*

THE activity even of spatially fixed growth zones may fluctuate with season and for other reasons, so that growth shows a distinct pattern both in space and time, but the temporal pattern may be as invariant from point to point in space as the spatial pattern is invariant with time. This is illustrated very simply in the following table of hypothetical growth rates at three points  $x_1$ ,  $x_2$ ,  $x_3$  at three successive times  $t_1$ ,  $t_2$ ,  $t_3$ .

	$x_1$	$x_2$	$x_3$
$t_1$	3	1	2
$t_2$	9	3	6
$t_3$	6	2	4

It is therefore best to reserve the term “spatiotemporal pattern” for instances where the spatial pattern changes in time—and therefore also the temporal pattern in space. In most of such cases the spatial shift is orderly and it is often repeated cyclically. It may be continuous or discontinuous.

A simple monocyclic pattern is seen in the pelage of man: hair appears progressively later on the chin and chest than on the head and continues to grow vigorously there below the balding pate. It does not turn grey in the same sequence, however. Perhaps the most important example of a monocyte is the antero-posterior sequence in the vertebrate embryo (see Huxley, 1932). A transverse progression is seen in the plumage of birds and the pelage of mammals (Rawles, 1955): the first row of feathers or hairs develops almost, but not quite, along the mid-ventral line and successive rows are formed alternately on either side of this. In passing, it may be noted that the first stripe on the zebra's forehead is similarly off-centre. A monocyte may be due to a single pulse of growth moving slowly through the system.

A simple kind of polycycle is seen in the alternating phases of growth in length and width in the body of Crustacea (Needham, 1937) and fish (Brown, 1946), in the wings of insects (Waddington, 1950) and in other organs. The regular shift of 90° in the orientation of cleavage planes in early embryogenesis is a similar pattern, involving all three dimensions. These are not spatio-temporal rhythms of the type envisaged at the beginning of the chapter because there need be no change in growth rate at any one point, but only a change in

its direction and there need be no significant spatial variation in rate at any one time. In the simplest case, therefore, it may have neither spatial nor temporal patterning. Nevertheless, it shows a regular temporal variation in length/width/depth growth which amounts to a simple spatial patterning. The shifts in spatial pattern are discontinuous but in fact there is spatial discontinuity between the cycles of some other polycycles also.

This regular 90° shift in the direction of growth is an important pattern biologically since it is the simplest method of maintaining uniform growth throughout a three-dimensional mass without causing distortion (p. 32). It is an extension to two and three dimensions of the principle underlying the budding growth of some rhabdocoelae (p. 56). An important proviso for the extension is that growth must be virtually entirely in one direction at any one time and evenly distributed over the plane perpendicular to this direction.

Polycyclic spatial shifts in growth activity are seen in the reproductive organs of female mammals. In the uterus mitotic activity starts at the base of the glands, and travels to the top once in each oestrous cycle. By contrast, it starts at the surface of the decidua and progresses downwards to the muscular layer (Allen, 1942). These waves of activity probably differ from the monocyclic waves of early ontogenesis only in being periodically repeated; in both cases the evoking factor, hormonal in the reproductive tissues, moves slowly through the body or organ, so that a temporal pulse becomes a spatiotemporal wave.

One of the most striking of spatiotemporal waves (Fig. 5.1) is that in the pelage of rodents (Haddow *et al.*, 1945; Wolbach, 1950). Its pattern varies somewhat in the different taxonomic groups. In the mouse the main pattern starts in a zone along each flank and moves dorsal- and ventralwards, to reach the mid-line in about 35 days. There follows a rest period of about the same duration and then the next cycle starts in the original places. The phenomenon was first dramatically revealed by brief administration of a pigment which happened to become deposited in growing hairs. It has further interesting features; the spatial progress is not uniform, that is its rate fluctuates, and the peak rate of growth is not identical at each point but is greater on the back and belly than on the flanks. It is a discontinuous pattern, at least spatially, since a series of pulses start always from the same points in the same direction, and do not travel full circle. Each wave is accompanied by one of vascular dilatation and of enhanced oxygen consumption. Rather surprisingly (p. 352) it is not affected by denervation. There is a regular cycle of activity in the individual hair follicles of man but no orderly polycycle has been recognized in his pelage as a unit.

Another type of spatiotemporal pattern is the phyllotactic, so called because it is seen most characteristically in the order of formation of new leaves on a shoot. The new items are discrete, and therefore growth is spatially discontinuous, but they do appear in regular succession, following a continuous spiral path along the stem of a plant or the body of an animal. In *Hydra* new buds appear along the spiral with a radial angle of 120° between successive members. It is therefore said to have a phyllotactic ratio of 1/3, three buds in

each turn around the body, the  $n$ th bud always being vertically above the  $(n-3)$ th. The possible phyllotactic ratios are members of a Fibonacci series:  $\frac{1}{2}, \frac{1}{3}, \frac{2}{5}, \frac{3}{8} \dots$  both numerator and denominator of each term being the sum of those of the previous two terms. Tracing successive members of this series an observer makes progressively more turns of the spiral and passes a larger number

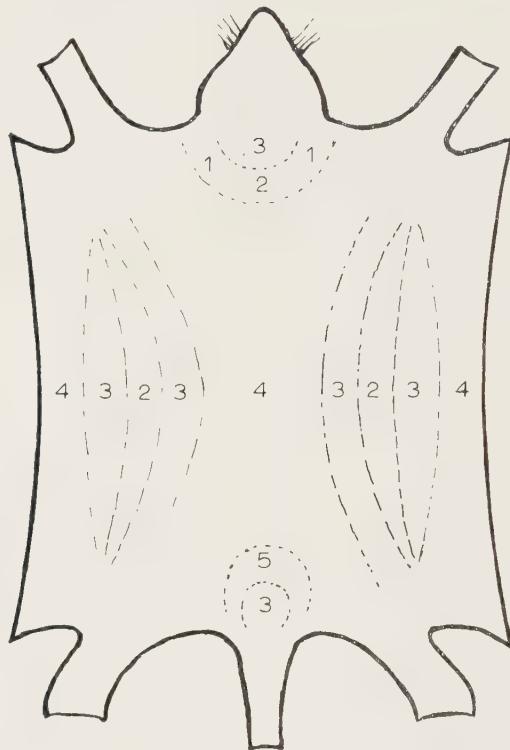


FIG. 5.1. DIAGRAM OF THE PELT OF THE MOUSE OPENED ALONG THE  
MID-VENTRAL LINE AND SPREAD OUT

The numbers in order show the origin and spread of a spatiotemporal pulse of growth. The main pulse starts along the flanks and moves dorsally and ventrally.

of buds between any two which are vertically in line; for leaves and some other structures it is important that there shall be minimal superimposition and therefore a high phyllotactic ratio.

The limiting ratio of the Fibonacci series corresponds to an angle of  $137^{\circ} 30' 28''$  between successive members and it is interesting that this is the angle actually found (within the limits of accuracy of the measurements!) between successive new tentacles inserted round the margin of the bell of the medusa, *Gonionemus* (Komai, 1951). Here the spiral has been telescoped down to a circle so that exact superimposition must be avoided at all costs. Actually four

cycles of tentacle formation are in progress simultaneously, one in each quadrant of the bell, so that the angle between neighbouring new tentacles is a quarter of the above critical angle.

The transverse bars of pigment in feathers (Fig. 4.15) are due to a temporal rhythm, but in a spatially uniform and virtually invariant zone (p. 48). In some birds, however, a transverse series of spots replaces this bar (Hardesty, 1933) and so both spatial and temporal rhythms exist; moreover in a simple way each varies in the dimension of the other, and the pattern is a true spatiotemporal one. Similarly the shells of some snails have transverse bands of colour,

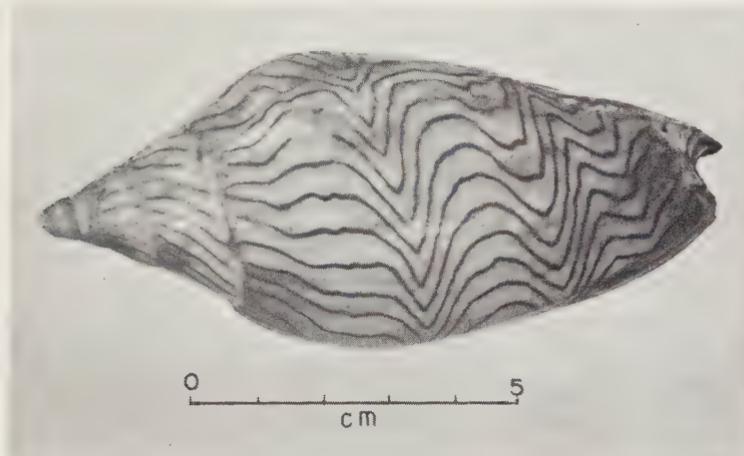


FIG. 5.2. SHELL OF *Voluta undina*, A MARINE GASTROPOD, TO SHOW THE PIGMENTARY PATTERN, FORMED AS DESCRIBED IN THE TEXT

(Selected by J. Hull; photo: P. L. Small)

again due to a temporal pattern of growth while others, such as *Cepaea*, have longitudinal bands, that is to say a spatial pattern with no temporal variation. Others such as *Voluta* (Fig. 5.2), have a spatial pattern which changes with time, giving a very striking spatiotemporal picture. The pigment is deposited by the mantle edge and incorporated into the growing shell (p. 174). Deposition begins at a number of points spaced along the mantle edge and as the edge itself grows forwards the activity moves from these points both ways along the mantle edge until it meets that of the adjoining zones, when they mutually extinguish each other. A new phase of deposition starts at the original points on the edge and a series of parallel zig-zag lines results (Comfort, 1951). Some molluscs have even more complex patterns, but this one is ideal in its regularity and relative simplicity.

### 5.1. Spatiotemporal Patterns in Embryogenesis

In principle it should be possible to give a complete account of the development of any animal in terms of the patterns considered in this and the two

previous chapters, supplemented by some morphochoresis in early stages (Dalcq, 1960). Such a complete description of the growth pattern throughout the four dimensions would be extremely valuable and interesting. The examples already studied algebraically (p. 37) and geometrically (p. 33) have been relatively simple by the present standard, or have been limited to the post embryonic period, or both. During embryonic stages patterns are more complex, particularly during periods of topological change, such as gastrulation and neurulation.

By contrast to the extensive knowledge of regional differentiation in embryos, little is known of their growth patterns. There are several reasons for this. In the first place, differentiation changes are the more easily recognized and described, or perhaps it would be more correct to say that the recognized changes are more easily described qualitatively, as differentiative processes, even though, in fact, they may be due largely to differential growth (p. 2). Secondly, topological changes and other movements of cells complicate the interpretation of size and shape changes. Thirdly, a reliable and consistent index of growth is not easily found; mere size increase may be misleading when it is so extensively due to water uptake (p. 266). The rate of cell division is often misleading in early stages because it merely divides up an egg which is not yet growing; in the later stages when it is reliable its use is a very time-consuming method.

Some embryos grow from the very first cleavage (Baker, 1948), by the intake of external material, and this is particularly true in viviparous animals. The blastula of the scyphozoon, *Chrysaora*, grows seven to ten times in linear dimensions (Berrill, 1949b) and the parasitic embryos of the Narcomedusae also grow extensively. Stauromedusan planulæ may grow and bud repeatedly, and similar embryonic processes are common in other coelenterates and in platyhelminths. The chick blastoderm grows three times in diameter during the first ten hours of development (Witschi, 1956, p. 231). In cases where cleavage is a mere partitioning, true growth begins at a variable stage and rate, depending on the amount of food stored in the egg. If there is little food the embryo must differentiate such essential organs as the gut and must feed before it can grow. Once it does start the rate is soon maximal (p. 12). The amount of growth as an embryo has increased progressively in ascending the vertebrate phylogenetic series (J. Needham, 1931, p. 440), correlated with a lengthening embryonic period and, in turn, with increasing food provision; no doubt this may have been one factor predisposing some of these animals to neoteny, that is to complete their growth while still in a very immature stage of differentiation.

Cell proliferation is usually most rapid at the animal pole of the blastula and this persists as a centre of ectodermal expansion, the animal pole cap. A wave of accelerated cell division progresses from here back along the dorsal line of the embryo of Amphibia following, and probably driving backwards, the dorsal lip of the blastopore. This area of proliferation is the axial rudiment, the future neural tube, and it has a high rate of proliferation in the trout also (J. Needham, 1942, p. 329). Its activity probably continues back directly as the growth zone

of the tail bud, an outstanding example of a spatiotemporal wave in an embryo, and possibly the basis of the general antero-posterior wave mentioned earlier (p. 62). It also contributes to the initiation of gastrulation at the dorsal lip and the invagination of an organizer region, forward under the dorsal surface. Here, and also in the process of regeneration, organizing power is correlated with growth and other physiological activity.

Proliferation in the general epidermis of the embryo is slower than in the dorsal axial tract, the future nervous system, but still considerably faster than in tissues derived from the vegetative hemisphere, and particularly than the yolk endoderm. The rate in the mesoderm is intermediate between those in the ecto- and endoderm (J. Needham, 1942). By the seventh day, in the rabbit embryo, these differences have almost disappeared (Minot, 1908, p. 221), the rates per thousand cells of ecto-, meso- and endoderm, at any moment then being 18, 17, and 18 respectively. By the tenth day these rates have fallen to 14, 13 and 15 per thousand, now possibly indicating a slight superiority in the endoderm. The precocity of the ectoderm, which must meet the challenge of external factors, seems adaptive and is seen also in regeneration (Needham, 1952). By the thirteenth day of gestation, in the rabbit, average proliferation rates have fallen to 10 per thousand.

After the establishment of the main cell layers growth rates become increasingly differential within them and the average rates quoted above fail to detect this. The rate is low in such organs as the mammalian limbs which are not yet required for use and also in such organs as the mesonephros which are purely for temporary use. A high local growth rate marks the main phase of development of particular organs, for example the eye (*see* Bonner, 1952), in which the morphogenetic role of growth is particularly clear. The growth of specific organs is often better known than that of the earlier embryo as an entity, and it follows the kinds of patterns already described. The linear elongation of tubes plays a leading part in embryonic organogeny. There is a mosaic progression in development, that is to say growth becomes differential between ever smaller sub-units of anatomy, again well illustrated in the eye (Fig. 4.4). For further details Waddington (1956), Witschi (1956) and other textbooks of embryogenesis may be consulted.

It is evident that we have some idea of the general patterns of growth which are possible and can cite actual examples of each, but that a detailed, comprehensive picture of growth has been obtained in very few animals. This is due partly to the amount of labour involved and partly to limitations in our powers of description. It is a field which merits a dedicated interest.

## CHAPTER 6

### *Growth Processes involving Remodelling*

It was pointed out (p. 28) that degrowth in some form is a common component of normal development; naturally this will complicate the pattern of growth. Two main classes of degrowth were recognized, those in which a continuous small amount of remodelling is necessary with increase in size and those in which the whole anatomy changes or metamorphoses rapidly, in connexion with a radical change in the way of life. Although the latter is the more complex, it will be considered first because it is a more clearly defined phenomenon than other types of remodelling.

#### **6.1. Growth Patterns during Metamorphosis**

The most spectacular examples of metamorphosis, and the best known, are those of insects and Amphibia, but almost as remarkable in the extent of the change are those of the echinoderms and tunicates. Other interesting examples include those of the nemertines, polyzoa, phoronids, brachiopods, cirripeds and copepod Crustacea, and flat-fish. The extent of the change depends mainly on the duration and importance of larval life, the final size of the larva and the extent to which larval and adult modes of life have come to diverge. Many of the marine invertebrates have a brief larval life, not very different from that of the adult, and a small larva, so that the changes are not very great. In some of these, however, it seems to be economic for an adult "rudiment" to grow *de novo* from a small group of cells, and for the larval body to be discarded subsequently. This is in sharp contrast to the holometabolic metamorphosis of insects in which, although the adult develops from a large number of imaginal discs in various parts of the larval body, virtually all the larval material is re-used (Fig. 6.5).

The complete process involves the following components, and probably others: tissue disintegration (histolysis) with re-use of cells, cell disintegration (cytolysis) with re-use of materials, cell movements, cell proliferation and cell hypertrophy. All can be very differentially distributed in space. The control of such extensive metamorphoses poses a problem in some ways more difficult than that of embryogenesis, since many of the tissues become effectively fluid; on the other hand, the nervous system persists throughout and grows without any discontinuity (Power, 1952), and so is likely to be one important controlling factor.

Considering first the simpler examples, a very striking metamorphosis is that of the pilidium larva of nemertines (Fig. 6.1). Three paired and one or two

unpaired imaginal discs of epidermis grow in as the floor of invaginated sacs, which spread out under the general larval epidermis until they meet and fuse with their fellows on all sides. The discs, therefore, eventually form the whole

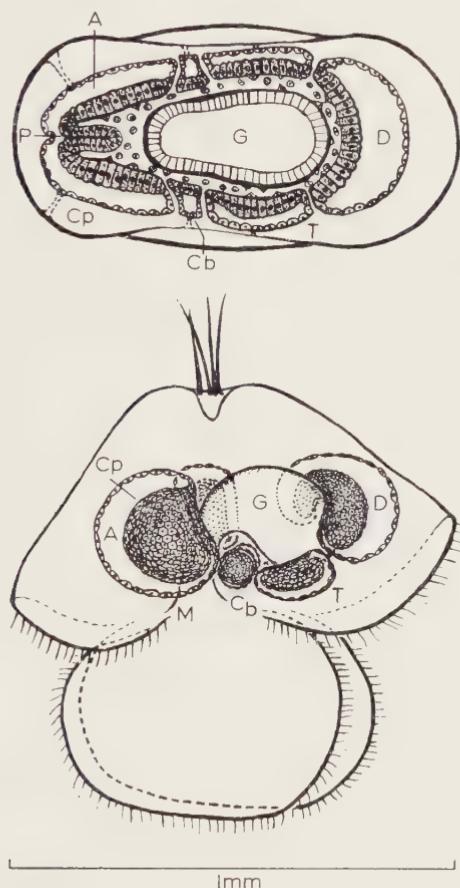


FIG. 6.1. DIAGRAMS OF (a) PLAN AND (b) LATERAL ELEVATION OF THE METAMORPHOSING PILIDIUM LARVA OF A NEMERTINE WORM, SHOWING HOW THE ADULT EPIDERMAL STRUCTURES ARE FORMED FROM THE INNER WALLS OF A NUMBER OF IMAGINAL INVAGINATIONS, THE IMAGINAL DISCS

A, amniotic cavity; Cb, cerebral disc; Cp, fused pair of cephalic discs; D, dorsal (median) disc; G, gut; M, mouth of larva; P, proboscis of adult; T, trunk disc.

epidermis of the adult worm, surrounding the larval gut and other persisting organs, and lying freely in an amniotic cavity contained, in turn, by the larval epidermis. The young adult eventually escapes from this, and abandons it. There is little remodelling. The proboscis and some other adult organs grow from special parts of the imaginal discs.

In *Phoronis* the epidermis of the whole trunk region of the adult grows as a large sac, the metasome pouch, again invaginated into the body of the larva. Like many insect discs this everts at metamorphosis and the essential viscera, as it were, herniate into it, giving the typical U-shaped gut loop of the adult. The larval tentacles and preoral lobe are cast off, but are ingested and not discarded.

Topologically the metamorphosis of echinoderms is probably the most complex in the animal kingdom. A bilaterally symmetrical, free-swimming larva gives rise to a pentamerous, sedentary or benthic adult, the antero-posterior axis of which does not correspond exactly with any main axis of the larva (Fig. 6.2). It is not even consistent in the different classes of echinoderm, if the external anatomy is used as the index. Thus although the vestibule which marks the oral face of the adult grows in as a sac on the oral face of the larva in crinoids, holothurians and ophiuroids, in crinoids it then moves to the posterior aspect and in the holothuria to the anterior aspect of the larva. In asteroids and echinoids (Fig. 6.2) the adult oral face develops on the left side of the larva. There is greater consistency in the five classes if the coelomic system is taken as the anatomical foundation: in all classes the coelomic sacs of the left side form mainly the coelomic structures of the oral aspect of the adult, while the posterior right coelom forms the main aboral coelom of the adult. The right anterior and middle coeloms form small enigmatic structures, or atrophy, while the left anterior and middle coeloms form the axial complex which extends from the anterior to the posterior side of the adult.

The vestibule of crinoids and holothurians moves from its initial position by differential growth, and the coelomic systems reach a position near the oral complex, likewise mainly by differential growth. The larval mouth and anus usually close and much of the fore and hind guts are resorbed; this and other material, such as the skeleton of the pluteus, is passed into the stomach and digested. The stomach persists and grows out into the adult fore gut (Fig. 6.2). The hind gut is an outgrowth of the stump of the larval organ. In most cases ectodermal stomodaeal and proctodaeal ingrowths meet these outgrowths and fuse with them. The second left coelom, the hydrocoele, has already begun to grow round to form the adult water-vascular ring, and the stomodaeum grows precisely through the centre of this. Outgrowths of the water-vascular ring form the rudiments of the first tube-feet and where they make contact with the epidermis of the vestibule this grows out to complete the podia. Unfortunately this material is not so readily available for experiment as that of insects and Amphibia, so that little is known about these remarkably organized processes. The vestibule, the invaginated oral face of the adult, resembles the imaginal discs of other groups of animal.

The metamorphosis of echinoderms is complicated by being two changes telescoped into one, the change from a swimming larva to a fixed pelmatozoon stage and then from this to the free living eleutherozoon adult. This is seen most clearly in *Antedon* among crinoids, but many asteroids temporarily fix in

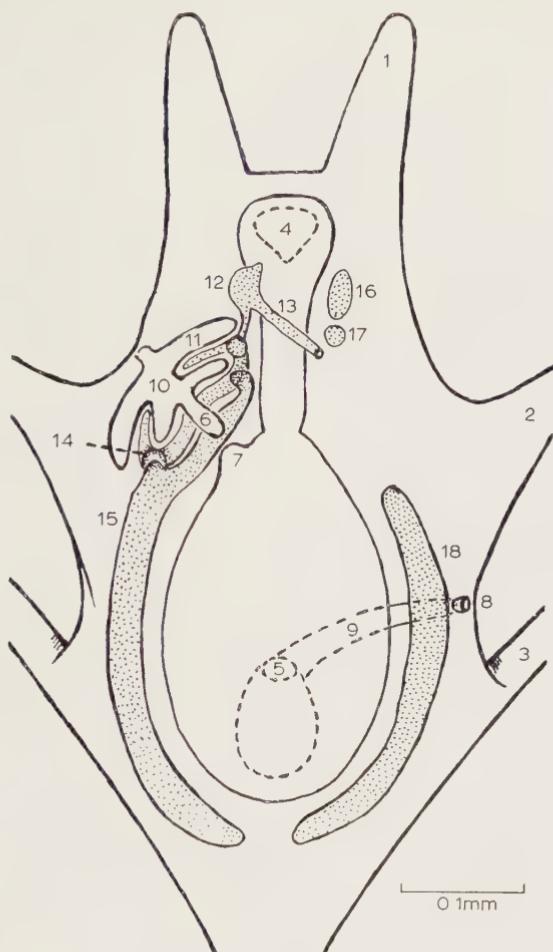


FIG. 6.2. DIAGRAM OF THE MAIN CHANGES AT METAMORPHOSIS IN A SEA URCHIN,  
AS SEEN BY TRANSPARENCY FROM THE ABORAL ASPECT OF THE PLUTEUS LARVA

A series of stages have been telescoped into the one diagram. 1, anterolateral arms of pluteus; 2, postero-dorsal arms; 3, postoral arms; 4, larval mouth; 5, larval anus; 6, adult stomodaeum; 7, adult oesophagus; 8, adult anus; 9, adult hind gut; 10, vestibule or annion of the adult "echinus rudiment"; 11, first podia of adult; 12, left anterior coelom (axocoele) of larva; 13, hydroporic canal; 14, left median coelom (hydrocoele) forming water-vascular system of adult; 15, left posterior coelom (somatocoel); 16, part of right axocoele, which atrophies in adult; 17, remainder of right axocoele, forming madrepore vesicle or dorsal sac of adult; 18, right somatocoel.

The right hydrocoele atrophies early.

the same way by their preoral lobe. This is often very large and is resorbed at the end of the fixed stage, though in *Luidia* the young star breaks free from it, as *Antedon* does from its stalk. In the other classes of Eleutherozoa, which no

longer fix at all, the adult rudiment nevertheless is largely restricted to the posterior half of the larva, and there is the same extensive resorption of the anterior part. Fixation necessitates rotation of the oral complex, as also in tunicates and cirripedes, but in the latter it does not result in asymmetry, which therefore may have some other significance in echinoderms. *Amphioxus* shows enigmatic asymmetrical growth processes which might be a legacy from an echinoderm ancestor.

For the reasons given, metamorphosis is much better understood in insects and Amphibia, although it is a complex of virtually simultaneous growth and regression processes. It is one of the most intriguing problems of metamorphosis that one set of conditions can induce the two, side by side in neighbouring tissues. Administration of the thyroid hormone to larval Amphibia will precipitate the whole complex, so that the limbs and parts of the jaws are growing (Fig. 6.4) while tail, gills and other organs are being resorbed. Holometabolous insects become a veritable tissue culture of freed cells, contained in a persisting epidermal case of which stomodaeum, proctodaeum and other imaginal discs are invaginate extensions (Fig. 6.5).

Those larval tissues which are not directly or indirectly used as adult tissues, but cytolise, are then ingested by phagocytic cells and the materials in some way made available to growing tissues (Wigglesworth, 1954*b*; Witschi, 1956). In both groups there is great economy in the re-use of the material (D. M. Needham, 1931; J. Needham, 1942; Anderson, 1948), the only weight loss being due to the inevitable energy expenditure (p. 252). Although insect imaginal discs begin their growth at an early larval age (Henson, 1946; Wigglesworth, 1954*b*), and adult amphibian organs begin theirs before demolition of larval structures, there is no doubt that the materials from the latter are required for the final phase of rapid growth. Some larval muscles must remain functional for the movements of eclosion and these do not break down until after metamorphosis, therefore (Finlayson, 1956, 1960). As in echinoderms, the process is remarkably and precisely adaptive in all its details.

Resorption of the tadpole's tail is an orderly process, beginning at its base (Fig. 6.3); curiously enough that of the tunicate tadpole is said to proceed in the opposite direction. The gut of the frog tadpole shortens to one-eighth of the larval length, correlated with the change from a vegetarian to a carnivorous diet. The shortening of the Anuran tail involves a shortening of the nerve cord and the atrophy of several segmental ganglia. The last three vertebrae fuse to form the urostyle. The aortic arches shorten as the gills are resorbed. The changes in the skull and jaws (Fig. 6.4) are a complex of differential resorption and growth (Pusey, 1938), leading to a striking reorientation of the quadrate and other bones. The periodic moulting of the skin begins at this time, being an adaptation to terrestrial conditions.

The new growth, like resorption, affects organs in all parts of the amphibian body, skin glands and lachrymal glands, cyclids, and other minor structures. There is an increase in the number of ganglion cells in the retina and of fibres

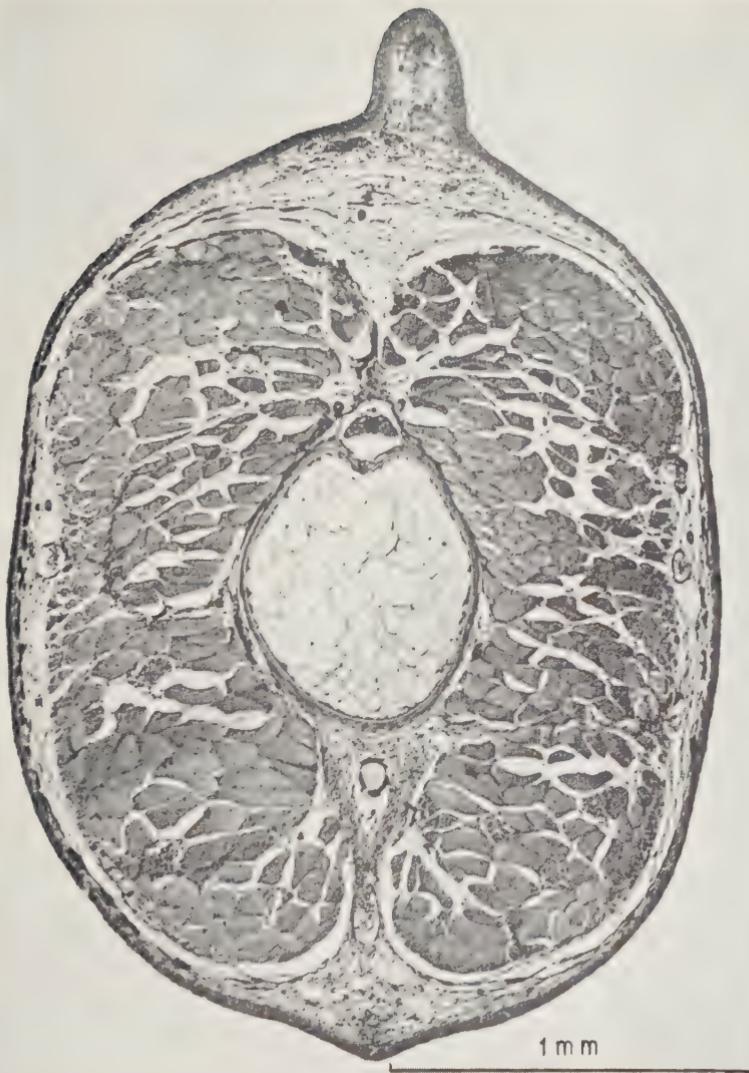


FIG. 6.3. PHOTOMICROGRAPH OF A TRANSVERSE SECTION NEAR THE BASE OF THE TAIL OF A FROG TADPOLE IN THE EARLY STAGES OF METAMORPHOSIS, SHOWING THE COMPLEX PATTERN OF DEMOLITION AMONG THE TISSUES OF THIS LARVAL ORGAN

The process is most advanced in the ventral fin, which has almost disappeared. Demolition is at its height in the mesoderm of the dorsal fin, as shown by the mass of rather resistant pigment tissue along its crest and by the thickened epidermis. Demolition of the latter appears to lag on that of the mesoderm. The regression of the muscle somites also is more extensive in the ventral than in the dorsal half, and is recognized by the nests of nuclei mixed with disintegrating fibre material. The reaction is simultaneous throughout the individual fibres, but the pattern of fibres affected seems rather irregular.

(Slide: P. A. Trotman; photo: J. S. Haywood)

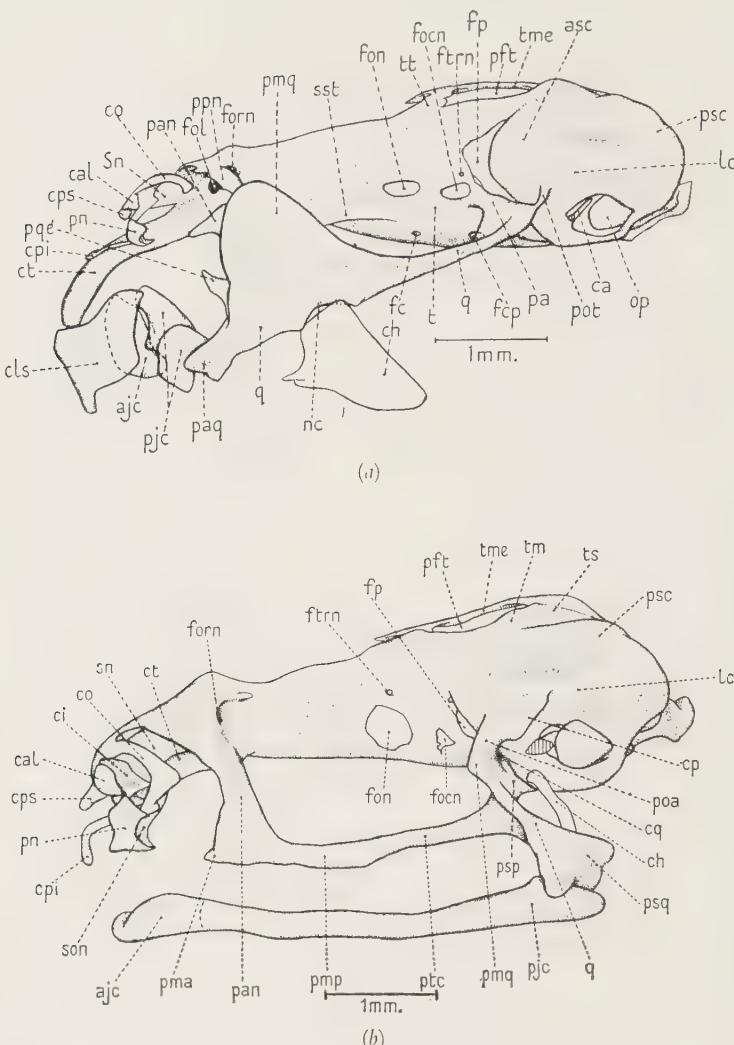


FIG. 6.4. THE SKELETON OF THE HEAD OF THE TADPOLE OF *Rana temporaria*, IN LATERAL VIEW, (a) EARLY AND (b) LATE IN METAMORPHOSIS, TO SHOW THE EXTENT OF REMODELLING

In two senses the whole process hinges on the quadrate cartilage (q).

(From H. K. Pusey, 1938, Quart. J. micr. Sci., 80)

in the optic nerve, and a corresponding growth of the optic tectum. The region of the mesencephalon, in which the ophthalmic branch of the fifth cranial nerve relays, similarly enlarges as its sensory supply becomes greater. Ossification now begins in many of the bones, in Anura and some Urodela. Limb growth is the most striking and important single component.

In insects there is a similar variety of growth processes (Imms, 1957; Wigglesworth, 1954*b*). The adult fore- and hind-guts usually grow each from an imaginal ring of tissue within the corresponding larval organs (Fig. 6.5) and the mid-gut develops from anterior and posterior portions of direct larval origin, together with two polar rudiments of newly condensed cells (Henson, 1946). Limbs, wings and other organs grow from imaginal discs, often deeply sunken as in other invertebrates. The sunken imaginal disc appears to be a structure not found in Amphibia, though the embryonic amnion of the higher vertebrates has some resemblance to it. The cells of an insect's imaginal rudiments are small, with diploid nuclei, and most of the specialized, polyploid larval cells are demolished. Some become diploid again, however, by rapid repeated nuclear division, and contribute to adult tissues. Some of the latter themselves become polytene (p. 134), later. The general epidermis is extensively formed from imaginal disc material in the walls of the peripodial sacs of the thoracic legs, and from superficial discs in the abdominal segments.

Although they can scarcely be more complex, some metamorphoses are even more profound than these. Ectoproctan Polyzoa upon settling become a mere sac of epidermis surrounding a mass of free cells, and the whole adult polypide develops as an ingrowth from the epidermis, around which mesoderm then condenses and proliferates. Cirripede parasites, such as *Sacculina*, degenerate to almost the same extent, within the mantle, which eventually contains little more than an epidermal sac of reproductive cells. This is perhaps the most extensive and degenerative metamorphosis known, apart from that of such copepods as *Xenocoeloma* (Caullery, 1952). At the same time the cirripede antennules acquire a remarkable power of growth with branching, to become rootlike absorptive organs. Some rhizocephalan cirripedes, such as *Thompsonia*, acquire the power to multiply the whole mantle complex; this is reminiscent of the asexual proliferation of some platyhelminth parasites.

At the other extreme are cases where metamorphosis is restricted to relatively few organs, or where the rate of change is too slight or slow to justify the term. Pubertal changes usually continue juvenile growth too smoothly to be called metamorphic, but in such instances as the second gnathopod of the male of the amphipod *Orchestia* (Charniaux-Cotton, 1949) the term would be justified. The carpal segment grows absolutely shorter (*enantiometry*) while other segments are growing in a normal fashion. These sub-metamorphic changes grade into the type of progressive remodelling to be considered next. It is probably reasonable to insist that a process cannot be metamorphic unless there are regressive components. On this criterion (Needham, 1952, 1960*a*) regeneration is metamorphic, its regressive and progressive phases possibly being rather more sharply segregated than in true metamorphosis; it often has the urgency of metamorphosis since the animal may be seriously incapacitated until it is completed. It may involve as much remodelling as metamorphosis, particularly the type of regeneration known as *morphallaxis*.

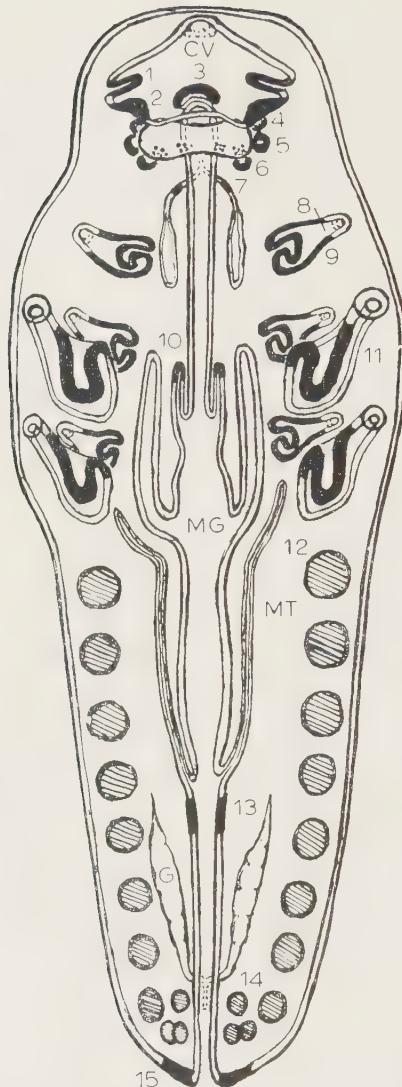


FIG. 6.5. DIAGRAM OF AN INSECT PUPA IN DORSAL VIEW AND BY TRANSPARENCY,  
TO SHOW IMAGINAL DISCS

The figure shows imaginal discs of: 1, antennae; 2, eyes; 3, labrum; 4, 5, 6, mouth parts; 7, salivary glands; 8, walking legs; 9, thoracic epidermis (peripodial membrane); 10, fore-gut; 11, wings; 12, abdominal epidermis (only one of the two discs on each side is represented in each segment); 13, hind-gut; 14, gonapophyses; 15, rectum. The mid-gut (MG) and Malpighian tubules (MT) are usually replaced from scattered nests of cells. CV, cephalic vesicle, housing 1 and 2; G, gonad.

## 6.2. Remodelling during the Course of Growth

It is not possible for some organs to develop by purely progressive growth, and this may account for the frequent occurrence of disintegrating cells and nests of cells, at all stages of development (Glucksman, 1951). In all organs it may be the way in which minor mechanical distortions (p. 32) are accommodated, but in some the regressive processes are systematic and highly organized and may have further significance. This is most easily recognized in the skeletal structures, where it is also most essential because of their limited plasticity. It has been described in parts of the mammalian skeleton, for instance by Sir Wilfred LeGros Clark (1958). The most interesting case is the growth of the head of the long bones (Fig. 4.17).

The first piece of remodelling in the long bones is the replacement of the initial cartilage rudiment of the shaft by bone. This begins by a direct but very brief calcification of the centre of the cartilage, possibly with no other significance than to facilitate, by strengthening, the next step, the invasion by blood vessels and osteoclasts. Together these remove the matrix by chemical and phagocytic action, again starting from the centre. This is possible mechanically because periosteal bone has already begun to form as a cylinder round the shaft (p. 59). A marrow cavity thus develops and it continues to enlarge throughout growth, both radially, keeping pace with the periosteal addition, and longitudinally, keeping pace with the activity of the metaphyseal disc which effects the growth in length (p. 54). This disc remains cartilaginous as long as growth continues, joining the ossified epiphysis to the shaft and proliferating tissue which eventually becomes trabecular bone (Fig. 4.17), light but anatomically well orientated for mechanical strength.

The most complex phase of remodelling occurs just proximal to this zone. It is necessitated mainly because of the great width of the head, so that it must be pared down when that region in turn becomes part of the shaft, during the further elongation. The wall of the bone is flared out like a funnel in the head region and the longitudinally orientated trabeculae are inserted into this. By using radioactive material, Leblond *et al.* (1955) have shown that these trabeculae elongate following up the activity of the metaphyseal cartilage and that material is deposited also on the inner face of the funnel, between the bases of the trabeculae. Meanwhile material is removed by osteoclasts from the outer face of the funnel, which therefore grows bodily distalwards. Since the shaft is also growing continuously in width, a particular point in the funnel, near its base may experience in turn trabecular growth, growth of the inner face of the funnel, resorption on the outer face of the funnel, growth in width of the shaft wall and finally resorption on the inner face of the shaft wall. It is therefore not surprising if histological sections (Fig. 6.6) show fragments of several generations of Haversian systems, intermingled.

The partial replacement of Haversian systems shown in this figure in fact is probably part of a more extensive and sustained type of remodelling. The remodelling of the head to become shaft tends to involve the complete removal

and complete replacement of material at any particular point rather than the piecemeal change shown in figure 6.6. Haversian systems are more characteristic of compact than of trabecular bone, and in any case they are not found

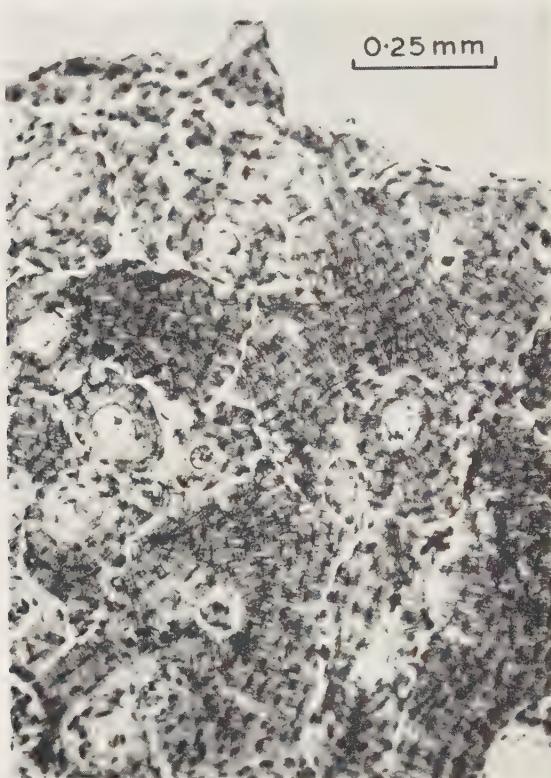


FIG. 6.6. TRANSVERSE SECTION OF A LONG BONE OF A MAMMAL, TO SHOW HISTOLOGICAL STRUCTURE AND, IN PARTICULAR, SUCCESSIVE GENERATIONS OF HAVERSIAN SYSTEMS, EACH PARTIALLY DESTROYING AND REPLACING PREVIOUS GENERATIONS

The concentric lamellae of bone, around central Haversian canals are evident; the small dark spaces lying in rings between the lamellae are the lacunae which house individual osteocytes. These connect with neighbours through the many fine canaliculae shown. Fragments of older generations of Haversian systems are seen filling gaps between the latest, most complete systems.

(Slide and photo: P. L. Small)

in all mammals (J. Currey, personal communication). They consist of concentric layers of bone and bone cells, laid down in order from the outside of a cylindrical space formed by a previous phase of bone removal; when the system is completed the lamellae have filled the space except for the central canal occupied by the blood vessel of the system. The full explanation of the repetitive Haversian replacement is uncertain, but changing stresses in the material due to growth processes outside the bone itself may be a contributory cause. Almost

every change in size and form of the body will change the distribution of stresses throughout the organ. In addition, bone matrix may be purloined in times of mineral shortage or of increased demand, and subsequently replaced (McLean, 1942).

The dermal bones of the vault of the skull also are remodelled, mainly by resorption on their inner face and extension on the outside (p. 59). Osteoclasts line one face and osteoblasts the other (Fig. 6.7). With the additional help of

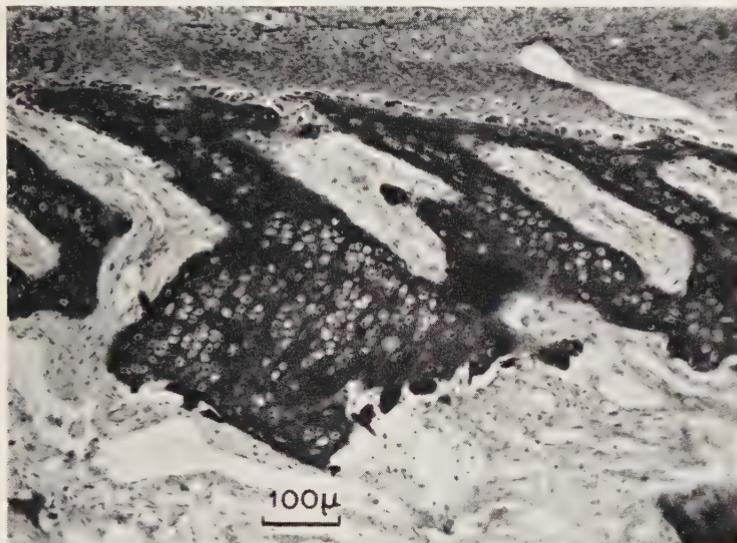


FIG. 6.7. SECTION OF CALCIFIED CARTILAGE AND SURROUNDING TISSUES IN THE NASAL BONES OF A YOUNG MAMMAL, SHOWING OSTEOBLASTS LINING THE SURFACES TOWARDS THE TOP OF THE PICTURE AND LARGE DARK OSTEOCLASTS ERODING THOSE TOWARDS THE BOTTOM

(Slide: R. Watt; photo: P. L. Small)

growth round the periphery (p. 44) this enlarges the cranial cavity to keep pace with brain growth. Pressure from the cranial cavity promotes resorption on that face. Pressure from the outside also will induce resorption and in periods when it was fashionable to wear heavy wigs skulls tended to be abnormally thin! Where the bones are excavated by air sinuses the remodelling is more complex.

A somewhat similar remodelling occurs in the corona of echinoids, a relatively rigid globe which grows mainly by the addition of new plates round the aboral pole (Deutler, 1926). This method of growth necessitates a change in curvature of existing plates, and a change in their superficial area and shape, particularly since the curvature is not uniform throughout, but is maximal below the equator and minimal across the oral face (Fig. 6.8). The remodelling depends partly on the growth round the margins (p. 44) of the plates and also

on the addition to the *inner* face (p. 59). The sites of deposition were revealed by the ingenious method of feeding algae for short periods, when their pigments were incorporated into new skeletal material. Deposition on the inside itself would tend to decrease the size of the cavity but from the familiar party problem of the band round the earth we know that quite a small circumferential addition at the margins of the plates will compensate for this; in any case, however, the addition of new plates aborally has a much greater effect in enlarging the cavity (Fig. 6.8). Deposition on the inside is important because this is

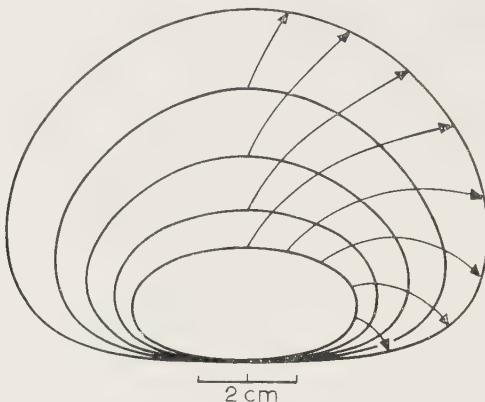


FIG. 6.8. SUPERIMPOSED OUTLINES OF MERIDIONAL SECTIONS OF THE CORONA OF A REGULAR SEA URCHIN AT SUCCESSIVE STAGES OF GROWTH

The arrows plot the trajectory of particular points on the meridian, and show that growth is mainly at the aboral pole. Their progressive increase in curvature with time shows that growth is maximal at this pole and is graded to a minimum at the oral pole.

the easier way of reducing the curvature, which is necessary as the size of the corona increases; it is also necessary because tissues are too scanty on the outside of the corona to permit adequate growth there. There is a regular gradient in growth activity from a maximum at the aboral pole to a minimum at the oral pole: here, in fact, there may be active resorption.

It is clear that remodelling is a very common and necessary component of growth. In regeneration, in the moult cycle of arthropods, and probably also in other cases there is a relatively sharp segregation of regressive and progressive periods but in most metamorphoses and other spectacular processes the temporal patterns of the two are as complex as their spatial patterns. Growth and degrowth may proceed in close proximity and a particular controlling factor may promote both, so that the individual tissues and organs must receive their more specific instructions through some other channel. The control of the whole process of metamorphosis is as complex and problematical as that of early embryogenesis. It is a field still offering great scope for further research, particularly at the histochemical level.

The morphogenetic details of metamorphosis vary greatly in the different groups of animal and the extent of the remodelling varies enormously but there is probably a common metabolic basis to all, which is also similar to that in regeneration and other types of remodelling. The individual organs and tissues probably have their specific instructions mainly from genetic and from earlier epigenetic sources (Wigglesworth, 1959). Probably some comes from neighbouring tissues, while systemic factors, hormonal and nervous, and external factors affect mainly the speed of the process as a whole. This picture may prove valid for other types of remodelling also.

## CHAPTER 7

### *The Growth of Organs*

THE organ is the first natural level of magnitude below that of the whole organism, and the main interest of the present chapter is in any new aspects of growth which emerge at this level. It is necessary first to look into the question of definitions, since organs vary a good deal in size and form: there is a large percentage of tissue in the body, the connective tissue in particular, which cannot be allocated to an organ in the usual anatomical sense. For the present purpose it would be better to use a physiological definition of the organ as a system of tissues serving a specific function; the connective tissue in its entirety may then be regarded as a discrete organ. The rationale for this is that functional hypertrophy, physiological regeneration and most other growth processes often affect the physiological organ as a unit, however diffuse it may be anatomically; the pelage and the whole skin behave as units, except after local damage or wear, and similarly the vascular bed, the connective tissue, and so on. Fortunately, anatomical organs are usually also good physiological organs, and there is no justification for a new term: the physiological organ is only a somewhat wider category than the anatomical. It is particularly necessary to recognize the diffuse organs since a great deal is known of the growth of such examples as the skin; at the same time it will be evident that they are uniform expanses of a particular set of tissues so that there is much about their growth at this level which could be studied equally well at the lower, tissue level. However, the spatiotemporal rhythms of growth in the pelage of rodents (p. 63) provide a good instance of a feature which would not be revealed by the study of a small isolated piece.

The diffuse organ in fact may prove to be something of a fiction. The epidermis, and the skin as a whole, vary in the different parts of the body and so does the connective tissue. It is not yet certain if the common properties of all connective tissues are more important than those which are specific to particular types of this general tissue, or to the connective tissue of a particular organ.

Physiologists also recognize a higher category, the functional association or *system* of organs, such as the alimentary and endocrine systems; in the last of these, certainly, there are important aspects of growth which concern the system as a unit (p. 383). For most growth purposes, however, organ systems are just collections of individual organs. The distinction between the two loses most of its importance in face of the great variation in size and character among individual organs, not only between animals but also within the same body:

the liver and a hair are each an organ for the present purpose, and the present interest is in any discrete functional part of a body.

The first aim is to see if there are features of growth which are characteristic of all such discrete parts, from the organ systems of the mammals down to the organelles of the Protozoa (Weisz, 1954), and which are not evident in the growth of the whole body. The second interest is in the differences in growth pattern between organs, and the third is in the interaction between organs, in the course of their growth, and between them and the body as a whole. Later it will be interesting to look for features which are size-dependent; this could be studied first on homologous organs in species of different absolute sizes, and secondly on organs in general, between animals which differ both in taxonomic group, that is genetically, and in body size. Comparisons have already been made between the growth rates of a metameric series of homologous organs (p. 37), and some other features of organ growth have been considered in passing. Obviously the spatial patterns of growth in the body depend extensively on the contributions of the individual organs.

The growth of organs is relatively easily studied *in situ*, by both direct and indirect methods, in contrast to the difficulty at lower grades of magnitude. Careful measurements of the size of many of the organs of mammals in relation to age and body size have been made by Latimer and others, mainly in the journal *Growth*. It is also possible to remove some organs or parts of them and to test the effect on the growth of the remainder. Particularly in the lower vertebrates and invertebrates, it is possible to transplant organs bearing a suitable label into other individuals, sometimes of other species, though this is limited by the phenomenon of immunity reaction. Parabiotic union of two individuals also has been a useful method, when one of them has an organ pathologically or otherwise labelled. Many of the small organs of embryos can be grown *in vitro* (Fell, 1953), and by skilful technique some success has been achieved even with larger, adult organs (Carrel and Lindbergh, 1938).

The growth of individual organs (Fig. 4.1), like that of the body as a whole (p. 13) gives a sigmoid curve of size against time and some, e.g. the reproductive organs, have two postnatal cycles of growth. The nervous system has only one, and its pre-inflectional half is prenatal. The human thymus shows also a sigmoid curve of degrowth, at the end of childhood (Fig. 4.1) and this can be regarded as true physiological degrowth with a certainty which could not be accorded to the senescent degrowth of the whole body (p. 30). These organs (Fig. 4.1) were chosen for illustration because of their special features. Most organs have curves more similar to that of the body as a whole, though differing in detail. The growth of particular organs relative to that of the whole body often shows a good approximation to simple allometry (p. 35) or to some other simple relation. In some cases there are good physiological reasons for this but in other instances the significance is uncertain.

One organ may respond to a factor which has no effect on the growth of

others, so that at the organ level interest moves from general to specific factors. A deficiency of calcium or of vitamin D stimulates growth of the parathyroid, and iodine deficiency causes thyroid hypertrophy. The increased use of an organ causes that organ alone to hypertrophy while regeneration and compensatory hypertrophy also are restricted to a specific organ, or to its homologous organ. Physiological regeneration, or maintenance, in the adult likewise varies greatly between organs, depending on the kind and intensity of wear on the organ. The differences are possibly further enhanced because each organ produces its own specific autoinhibitor (p. 356).

Differences in nutritive requirements have been detected *in vitro* (Carrel and Lindbergh, 1938), and Spratt (1958) found both quantitative and qualitative differences in the requirements of the various organs. The ovary is outstanding in its tolerance of a poor diet and the nervous system is very resistant to starvation, as it is *in vivo* (p. 29). The very variable resistance of the different organs *in vivo* therefore may imply equally varied growth potencies. Competition among organs is an additional factor *in vivo*. The growth of the oyster's shell almost ceases while the gonads are maturing (Orton, 1928) and epidermal warts in mice cease growing while the pelage locally is in the phase of maximal growth.

Notwithstanding these differences the growth of organs is well coordinated and orderly, and this has been seen already in such instances as the series of limbs of the centipede (p. 37). The general coordination may be resolved into a number of particular correlations. The importance of closely correlated growth in the upper and lower jaws of vertebrates has been stressed by occasional abnormalities such as that of the bulldog. The dentine and enamel layers of teeth (Fig. 4.16) must be laid down in contact and in step, by two independent germinal matrices (p. 51). The growth of a snail's shell keeps pace with that of the body, though the exact pattern of growth in the various organs may be different from that of the shell and mantle. Muscles must grow to keep pace with the bones on which they originate and insert, although their actual modes of growth are very different.

Bones grow in the way already described (p. 77), but skeletal muscles initially by their cells increasing greatly in length and diameter and becoming coenocytic fibres, as large as  $40 \times 0.1$  mm in size. There is a limit to the useful length of a muscle fibre, however, so that the organ later grows in length mainly by the fibres sliding past each other (Meara, 1947), some being inserted into the tendon of one end and some into the other; the free end of each fibre is inserted into the connective tissue framework which binds and permeates the whole muscle. In large animals many of the fibres are inserted into this framework at both ends, and relatively few reach the tendons. New fibres are recruited at the same time (Davison, 1956); in the early stages at least this is mainly by fission of the coenocytia (Picken, 1960). In the *radialis* muscle of the rat there are 5900 fibres at birth, 7600 at 30 days of age and 8000 at 420 days (Ott, 1937). It would be interesting to know more of the pattern of recruitment.

Occasionally the correlated growth of organs fails temporarily, and this emphasizes the normal precision of the phenomenon. The keratinous epidermal scutes of the carapace of young tortoises may grow faster than the bony dermal plates below and in consequence are temporarily thrown into ridges. These are later ironed out by the increasing growth of the plates (Thompson, 1942, p. 173). In the hydroid *Obelia* the time of maximal proliferation in the endodermis at any point does not correspond precisely with that of the ectodermis, and again temporary folding occurs (Berrill, 1953).

In some cases a direct control of the growth of one organ by another can be detected, for instance of the cranium by the brain. This is even more evident within an organ: if the periosteum becomes torn a bone may develop large exostoses at that point. In a similar way the dermal papilla restrains the growth of the epidermal component of a hair follicle (Rawles, 1955). The lens and eye cup, and other instances are discussed later (p. 350). These interactions are found at all levels of organization and are both promotory and inhibitory. During ontogenesis the hierarchy of levels of interaction manifests itself also as a temporal sequence, the main organizer initiating the suite.

Interactions of this kind demand that each organ should respect the integrity of others. They may be closely apposed or even fused but they must not invade. *In vitro* most of them continue to respect their neighbours but the gonads are exceptional (Carrel and Lindbergh, i.c.); they will even invade the tissues of other species. The embryo's trophoblast behaves very similarly (Kirby, 1962). In this they resemble cancerous tissue (p. 95). Abercrombie and his school have investigated the basis for this at the cellular and tissue levels (p. 92).

Many organs show their own particular features of growth, but these do not all contribute to the general analysis at this level and space does not permit a detailed consideration of them. The wing of *Drosophila* may be singled out, since it illustrates growth in a small organ, has been analysed in detail (Waddington, 1950) and proves particularly interesting. It is interesting mainly because of the great orderliness of the whole process. There are two phases of cell proliferation or hyperplasia, each followed by one of cell enlargement or hypertrophy. As already implied by the alternations of length and width growth (p. 62) all the mitotic figures are orientated along the wing axis in the first phase of hyperplasia and transversely in the second. The hypertrophy following the second has itself two phases: in the first of these the cells extend mainly along the wing, and in the second across it, with some overlap between the two. Distension of the haemocoelic spaces with blood also contributes to the size increase and there are other components, not strictly growth processes. No doubt many other organs would show equally interesting patterns. Bonner (1952) has described in some detail the growth of a more complex organ, the vertebrate eye. The sclerotic bones of the bird's eye have three successive phases or pulses of cell proliferation (Hale, 1956) and this may be a very common feature of organ growth. These pulses offer a possible basis for some of the growth rhythms having a period of one to a few days (p. 19).

Physiological regeneration could most conveniently be considered at this level. An adult organ may grow continuously and relatively rapidly without becoming any larger, but merely replacing losses by wear. To make good their particularly heavy wear the incisor teeth of rodents grow 2 to 4 mm per week, compared with  $30\mu$  for most teeth even during their phase of maximal growth. It is a point of interest, and perhaps of real significance, that our finger nails grow at about the same rate, 2 mm per week, though the toe nails grow more slowly. Hairs on the legs also grow about 2 mm per week, and again their average length does not increase, but in this case the growth may be in new hairs to replace old ones which are shed, rather than growth to balance wear in the individual hair.

The epidermis of the skin and oesophagus (Fig. 4.24) is continuously replaced by cell proliferation in the basal or Malpighian layers. In the skin the products become cornified to withstand mechanical and chemical wear; they progressively dry and scarf off in superficial flakes. The epidermis of the palms and soles is completely renewed about every 19 days. This rate of production is maintained throughout life and may even increase in the elderly (Dublin, 1952). During a life time of 70 years the total thickness of epidermis produced will be about 1.3 metres, since the thickness of the plantar epidermis at any moment is 1.27 mm, of which perhaps 0.27 might be allowed for the thickness of the germinative layers. Elsewhere the steady state thickness and the rate of production are less.

*In vitro* organs appear capable of growth on a qualitatively poorer medium than smaller masses of cells, and a diet rich enough for the latter induces the more disorderly type of growth (Wolff, 1952) characteristic of tissue cultures (p. 88). Organs do not dedifferentiate *in vitro*, as a condition of growth, but rather their differentiation increases. Their qualitative requirements are more modest probably because they have greater powers of synthesis, but their quantitative needs naturally are greater, in proportion to their mass. Starvation arrests the growth of organs more quickly than that of small pieces of tissue (Spratt, 1958), and causes them to disintegrate.

It may be concluded that organs vary considerably in their pattern and timetable of growth, in their nutritional requirements, and in their power to resist starvation and other inhibitors of growth. At the same time they are interdependent in ways which lead to orderly, proportioned growth and which include both the promotion and the depression of growth in each other. Differences in the precise modes of growth of topographically associated organs does not prevent their increments, or at least their final sizes, from being exactly proportioned. This is one of the marvels of epigenesis, and also another example of end results which appear simpler than their means. Each organ normally respects the territory and integrity of others.

Small organs have a geometrically very regular mode of growth, based directly on growth at the cellular level. That of larger organs inevitably appears to be less orderly, though in fact it may be only less simple. On the

other hand, the larger the organ the greater its ability to fend for itself nutritionally, and to maintain its organization. This ability is not correlated simply with size, that is to say large organs are generally also the most highly differentiated and large animals the most highly evolved. The differences are continuously graded and at no point in the scale of size is there a sharp discontinuity in the growth properties studied. The analysis therefore may be carried forward to the tissue level.

## CHAPTER 8

### *The Growth of Tissues in Metazoa*

As indicated in the previous chapter the distinction between organs and tissues is not always very clear, and it is only certain that the present interest, following the general plan (p. 7), is in smaller masses of cells, of fewer types, than in the previous chapter. The heart, brain and muscles show that large organs may consist of a single type of cell, apart from the connective and other non-specific tissues which permeate all organs. On the other hand, quite small fragments of such organs as the kidney and eye may have a great variety of cell types. The interest here is in small masses rather than in purity of cell type, therefore; it is in populations of cells rather than in their organization.

#### **8.1. Tissues *in Vitro***

At present most of our knowledge of growth at the tissue level comes from isolating pieces of tissue *in vitro*. Conditions therefore are somewhat artificial but the abnormal, whether spontaneous or experimental, can throw light on the normal, and much has already been learned by this valuable, if exacting technique (Willmer, 1958). Even isolated cells, of various tissues, can now be cultured (Puck, 1957).

In the lower Metazoa, sponges and coelenterates, small masses and even isolated cells will survive, aggregate, reorganize and grow, simply in their normal external medium. Animals of higher grade demand a medium approximating to their internal environment. Tissues of the snail survive and show some of the activities associated with growth, even in unsterilized Ringer solution (Gatenby *et al.*, 1933, 1934). Arthropod and vertebrate tissues, however, demand strict asepsis, which indicates that the animal itself maintains sterile conditions *in vivo*: this is known to be true from haematological evidence. Given a suitable medium and the necessary nutrients, the tissues even of birds and mammals will grow and proliferate indefinitely, by the repeated subculture of fragments. Among the tissues tested those of Arthropods are the most difficult to culture (Goodchild, 1954; Bucklin, 1953), but they have now been induced to proliferate (Jones and Cunningham, 1961).

The classical experiments of Carrel, using chick heart fibroblasts, showed that the cells are potentially immortal, and at least that they can continue to grow vigorously after their owner has died of old age. Senescence, therefore (p. 24), and loss of the ability to grow is not primarily a cellular phenomenon. Adult plasma in fact has been shown to contain inhibitory factors (Medawar, 1940) and there is much further evidence from the cancerous type of growth

(p. 95) that the metazoon cell retains a greater power for growth than it normally shows *in vivo*, and that the normal control of this is systemic. At the same time the cells themselves *in situ* progressively lose the power to resist inhibitors of growth, as the body grows older (Medawar, 1940), a good example of double assurance (p. 343). They also take progressively longer to start growing when first explanted. They appear to have gradually acquired, or lost, something *in vivo* which can be reversed *in vitro*.

The cultured explant must be small enough to dispense with a circulatory system for its oxygen supply, and a 1-mm cube is near the limiting size. It has already been seen (p. 83) that even entire small organs from embryos may be cultured if they are below the limiting size, that their growth is orderly and that their differentiation increases. Small pieces of a tissue, however, show loss of differentiation, at both tissue and cell levels, in inverse proportion to their size. Growth activity is proportionately increased; this inverse relation between growth and differentiation is seen also in normal ontogenesis (p. 433). After its initial changes a culture *in vitro* in fact passes through a simple pseudo-ontogenetic cycle of decreasing growth rate, accompanied by redifferentiation. In iris epithelium (Fischer, 1946) the situation appears to be simply that, at first, cell growth and proliferation outstrip the production of melanin granules, which is the index of differentiation here.

The curve of size of culture/time has the familiar sigmoid form, with gross growth rate maximal around the time when the curve inflects. In these cultures, particularly from older individuals, there is a lag period, before any cell division or evident growth occurs; after this period of conditioning, or of escaping from previous inhibitory factors, the growth rate is immediately maximal, typical cells dividing every 12 to 24 hours; as in normal ontogenesis the specific growth rate is maximal near the outset. The total cycle is relatively short, a matter of days even if the medium is renewed, so that the tissue must have a strong intrinsic tendency towards growth limitation. That it is a property intrinsic to the tissue itself is shown also by the facts that, if a fraction of the culture is removed at the stationary stage it is regenerated quantitatively, and that there is repeated further growth on subculturing small fragments. Moreover, two explants in a particular volume of medium give a bigger harvest of new growth than one, while a new piece of tissue will grow well in the medium vacated by one which had reached the stationary stage. If left in the same medium a culture may be kept alive, but stationary, for at least three months. This may be compared to the adult condition *in vivo*, and the whole response is a model also for the cycle of events during regeneration. Injury or loss, in fact, must lead to conditions somewhat resembling those *in vitro*.

Apart from this light on the control of growth, tissue cultures have given much information on the nutritive requirements for growth in individual cells, and on the differential needs of the various types of cell. The first have proved largely non-specific to growth at this particular level but certain more peculiar features have been detected. For instance, pieces of mammalian tissue can grow

without added cholesterol whereas isolated cells demand it (Puck, 1957). This may be one of the reserve nutrients contained in relatively large tissue masses, possibly synthesized intercellularly, therefore. Isolated pieces of tissue demand all the nutritive factors required by the intact organism (White, 1954) as well as a large number of others (Willmer, 1958, p. 49). The latter are normally supplied by the whole organ (p. 87) or by such central organs as the liver. Consequently the best growth is produced by adding to the culture-medium extracts of whole embryos, not necessarily of the same species, together with adult blood plasma. The latter has complementary properties, perhaps due to certain products of hepatic activity. There is some growth on a simple mixture of pure vitamins, amino acids, sugar and salts but this is limited to the replacement of wear and tear (White, 1954).

A further feature of interest is that tissues *in vitro* are relatively insensitive to many materials and agents which promote growth *in vivo*: nucleic acids (p. 323), many of the amino acids and of the vitamins (p. 303), and the anterior pituitary and steroid hormones (p. 365). Once more the implication may be that synthesis is initiated and controlled at a higher level, though biochemical and other studies (p. 330) indicate that synthesis occurs mainly inside the individual cell. Hormones are generally thought to act at the surface of the individual cell (p. 383), so that this insensitivity *in vitro* deserves more investigation. The agents in question must be tested in a medium which is adequate also as a control medium, that is to say it must not contain a source of these agents. Embryo extract, therefore, is ruled out as an ingredient since it contains almost everything a cell could desire. However, it is so difficult to induce growth in the complete absence of this extract that most experiments merely compare the effect of added amounts of an agent with those of unknown but smaller amounts in the embryo extract of the control medium.

Mammalian and avian tissues can be arranged in the following approximate order of increasing demand for nutrients to permit their growth *in vitro*: carcinoma, blood cells, epidermis, osteocytes, fibrocytes, muscle, and lymphatic tissue. This is based primarily on simple quantitative requirements, though qualitative needs tend to run in parallel. Thus blood cells (monocytes) proliferate best on plasma alone and epidermis makes some growth without embryo extract. On the other hand the mechanocytes (Willmer, 1945, 1960), that is the connective, skeletal and muscle tissues, require considerable amounts of this extract, as much as 15 per cent of the medium for osteocytes and chondrocytes, and as much as 40 per cent for muscle (Fischer, 1946). Nevertheless fibrocytes are able to proliferate on plasma alone when in the presence of monocytes, an important example of tissue interaction. This particular case may be significant in wound healing, *in vivo*, since there is a massive invasion of the wound area by blood cells. In general, nutritive demands are inversely related to the rate of growth of the tissue *in vivo*; this is highest in blood cells and lowest in mechanocytes.

In general the latter are also more sensitive to growth inhibitors than

amoebocytes and epitheliocytes. Thus aldehydes, surface active agents and other substances (Medawar, 1940) may be administered in concentrations which inhibit only mesenchyme. Tissues vary also in the ability of their extracts to support the growth of other tissues (Willmer, 1958). The order is not closely related, directly or inversely, to that for their own nutritive requirements and one outstanding example is the relatively high potency of nerve tissue. This probably reflects the importance of the nerve supply in the control of growth (p. 352), and it is significant that a number of the viruses, such as herpes and poliomyelitis, attack nerve tissue specifically.

A further feature illustrated from this type of work is the interdependence between tissues in their growth. This is an extension downwards of the type of relationship already seen at the organ level (p. 84). In addition to the fibrocyte-monocyte relationship mentioned above, intestinal epithelium demands the presence of connective tissue for its growth, and cartilage requires both perichondrium and connective tissue. The interdependence applies also to differentiation (Grobstein, 1954).

The work has also revealed an interrelationship between growth and cell mechanics, both static and dynamic. An outwandering of cells is the first and sometimes the only, activity of cell cultures *in vitro*, for this movement has a lower food requirement than growth. Typically, cells at the periphery move out radially and the culture comes to have a circular or spherical shape. The movement may be a necessary preliminary for cell proliferation, which is most rapid just behind the edge of the migrating mass, but migration is said to continue after proliferation has ceased so that it is not the limiting factor for growth, although cell migration and cell division show a high degree of correlation.

*In vitro* a high oxygen supply favours cell division more than cell migration (Medawar, 1948) but ascorbic acid (not to be confused with ascorbic acid) depresses both activities (Medawar, 1940); both are normally maximal at the same time (Fischer, 1946) and both are equally restricted in large explants. Both are depressed on the side of a culture facing a second explant. Of course the movements of daughter cells in the later stages of a cell division contribute materially to the total movement observed. Cell division itself is a motor activity with resemblances to other forms of movement (Weber, 1958) so that the correlation with migration is not unexpected.

Outwandering is accompanied by an increase in microsomal material (p. 146) in the cells, and other changes, but the exact causal sequence is still far from clear. There seems good evidence that proliferation is inhibited by cell contacts (Abercrombie and Heaysman, 1954; Weiss, 1959) and is favoured by the looser texture which will result from radial emigration; cultures of micro-organisms, for instance *Hartmanella* (p. 113), similarly show a slower growth rate when they become crowded enough to make contacts with each other. Fibroblasts at first emigrate as spindles and the texture is very open. Later their peripheral edge spreads laterally and each becomes triangular in plan, covering

a larger area. This filling in of available space may be a factor causing the cessation of growth in a mature culture.

As already indicated (p. 85) migrating cells respect the territory of others and normal cells fill all gaps but rarely infiltrate (Abercrombie and Heaysman, 1954). Growth and movement are repressed on the opposing faces of two cultures growing in the same vessel. Nerve fibres are in a special category since they must normally make contact with many, if not all, cells of the body. *In vivo* they tend to penetrate tissues though they do so *via* available highways, along with the blood supply (p. 122); *in vitro* they differ from other types in crossing the gaps between separate culture masses (Weiss, 1959). It seems likely that the endothelial cells of the capillary walls and also connective tissue cells experience a similar general attraction.

Cells of other types, when mixed experimentally, tend to sort out into groups of pure type, so that there is an attraction for isologous cells and a repulsion for other types. Cancerous cells lose these properties along with other specificities. Finally there are specific bilateral affinities, as between ectoderm and endoderm at their junctions in the fore- and hind-guts. All these cell relationships are opportunist, normally adaptive properties.

The general lessons learned from tissue culture are first that cells are potentially immortal, with unlimited powers of growth and proliferation, so that their limited powers *in vivo* and their mortality depend primarily on systemic factors. These control the cells through the supply of nutrients as well as through more specific controlling agents. Secondly the work has shown that the growth of tissues and cells is affected by the activities, including the movements, of their neighbours and these purely local interactions are alone adequate to guide the culture through a stereotyped morphogenetic programme to a new steady state somewhat similar to that of a simple entire organism. This must be an important component of the control of growth *in vivo*. Thirdly it has been shown that each cell type differs in its nutritive requirements, its power of growth and of resisting inhibitors, and in other relevant features. These reflect (1) the extent to which the cell normally depends on synthesis elsewhere in the body, (2) its normal rate of growth *in situ*, and (3) the degree of its indispensability to the body in case of competition for limited nutrients. Finally, cultures *in vitro* have helped to confirm many facts about growth and about agents which affect it *in vivo*: in some cases it was essential that the facts should be checked *in vitro*.

## 8.2. Tissues *in Vivo*

A certain amount of information on tissue growth has been obtained by studies *in vivo* and these deserve more exploitation. Jane Overton (1955) examined the response of cell proliferation in the epidermis of Amphibia to fasting and feeding and to the stimulus of grafts of nerve tissue. The responses to fasting and feeding of course are indirect, and systemically mediated, and they provide useful information about this kind of indirect mediation. Cell

division does not cease until ten days after the onset of fasting and when feeding is resumed it starts after a lag of six days. Grafts of nerve tissue will induce proliferation even in a fasting animal, though their action also summates with that of food. Groups of epithelial cells tend to divide simultaneously (Lorrain Smith, 1932, p. 4): this may merely indicate a common origin, recently, from an active parent cell, as in cartilage (Fig. 4.2), or it may show that an active cell stimulates its neighbours (p. 422). There is now considerable evidence for the second possibility.

The blood cells, particularly of vertebrates, provide another example. Their proliferation is an instance of physiological regeneration (p. 5). Blood is commonly regarded as a tissue with a fluid matrix but the cells may also be compared with populations of microorganisms (p. 111), which are relevant at this level. Blood cells are ideal experimental material: they are easily recognized and counted, using very small samples of blood, and they are easily depleted or diluted *in situ* by bleeding the animal, and making up the fluid volume again. Bone marrow punctures and smears are routine techniques and the normal mode of production of cells in the marrow is now well known.

The restriction of blood cell production to the marrow cavity of certain bones is itself a feature of great interest: protection and economy of space are possible biological reasons for this. A further point of interest is that there is a progressive shift in the site of production during human ontogenesis, from the yolk sac or the placenta, in order, to the liver, the spleen, the marrow of the long bones and finally the marrow of the ribs and other small bones. Of course the liver retains its power of producing tissue macrophages, and the spleen of producing lymphocytes, and probably monocytes also; moreover the bone marrow has some activity even from an early stage, so that the shifts are relative rather than absolute. A strict requirement for a particular optimal temperature may be the determining factor (Maximow and Bloom, 1942). Lymphocytes and perhaps monocytes are proliferated in the haemal nodes and lymph nodes, as well as in the spleen, while all of the other blood cells, both red and white, are formed in the marrow sites.

The production of red cells has some interesting features, peculiar to a mobile tissue. The blood capillaries of the red marrow communicate with large sinusoid backwaters which may be completely closed off while the mother cells or haematoblasts are proliferating haematocytes. When these have matured the sinusoids open up and the cells are carried away into the general circulation. A parallel to this is probably seen in the early stages of regeneration in Crustacea (Needham, 1952) and Amphibia (Singer, *et al.* 1955) when the young blastema is effectively closed off from the rest of the body. The segregation of the testes of some mammals to an external scrotum (p. 418) is another instance where proliferation demands special conditions. The condition dictating the segregation is not necessarily the same in all cases. It is possible that the marrow sinusoids also function like those of the spleen, so as to regulate short term, but large scale, changes in demand for the cells. Certainly there is a

tendency for immature cells to appear in the general circulation following a heavily increased demand, due to haemorrhage or other causes.

There are  $27.5 \times 10^{12}$  red cells in an average human being, and they each have a life span of the order of 100 days. There are  $48.5 \times 10^9$  white cells with an average span of at most a few weeks (Gowans, 1959) so that around  $28 \times 10^{10}$

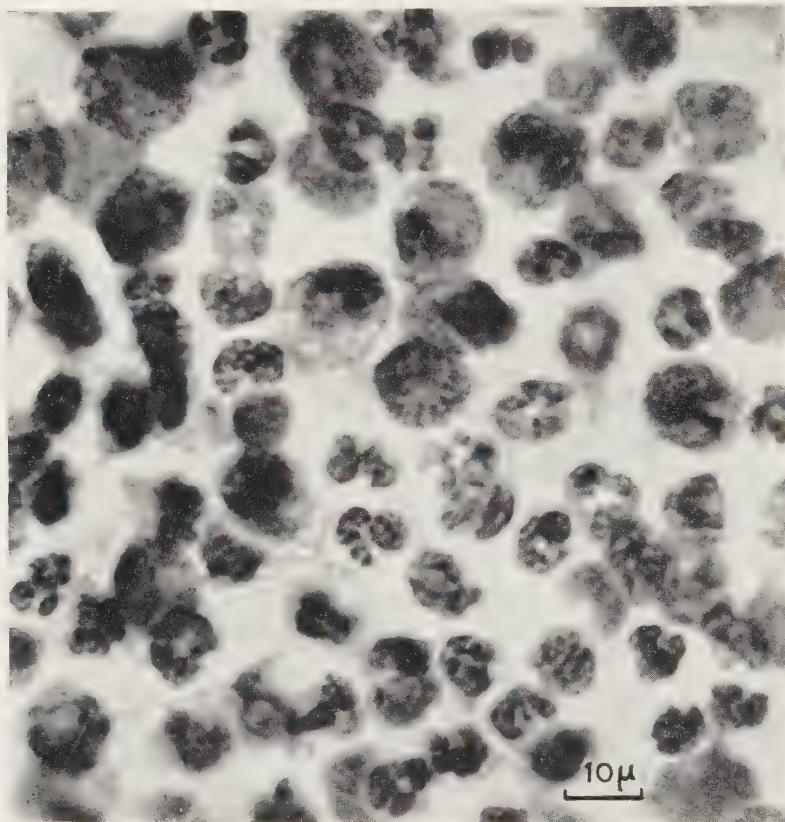


FIG. 8.1. BLOOD FILM FROM A LEUCAEMIA PATIENT, SHOWING NEOPLASTIC PROLIFERATION OF POLYMORPH LEUCOCYTES

There are also many immature cells present, normally not released from the bone marrow. The erythrocytes are only faintly stained.

(Slide: Dr. W. T. Catton; photo: J. S. Haywood)

cells are produced each day to make good losses; no doubt this is one of the major items of wear and tear in the body and amounts to as much as 20 grammes a day! Even in the dog, a smaller animal, as many as  $2 \times 10^8$  new lymphocytes are produced per hour, that is 55,000 per second! With such great normal activity it is not surprising that there is sometimes a deficiency in the number of red cells, one form of anaemia, or of white cells, leucopenia, and sometimes an excess of white cells, leukaemia (Fig. 8.1). Anaemia is one of the commonest

symptoms of vitamin deficiency but there are a number of types; some are due to a failure in haemoglobin synthesis rather than in cell production, though the two are not completely independent. Thus the haematinic principle (p. 315) and other agents which are necessary for haemoglobin synthesis prove essential in consequence for red cell production.

A great deal more is known about the physiological control of blood cell formation. One outstanding example may be mentioned since it is vital to the normal functioning of the red cells. This is the increased output of cells in response to anoxia, or chronic oxygen shortage, and also to an excess of CO<sub>2</sub> or of other acids in the body, which may be alternative signals of oxygen shortage. Blood cell production is very sensitive also to nutritional level, as in the other tissues (p. 92).

As already indicated (p. 82) a good deal of our knowledge of the growth of hair (Montagna and Ellis, 1958) and skin and other disseminated organs is relevant to tissue growth *in situ*. It is known that local proliferation such as that in a growing hair is accompanied by an intense accumulation of glycogen, as energy supply, and of materials specific to the tissue, such as the sulphur-containing amino acids. The speed of utilization of these stores is equally dramatic. There are local accumulations of nucleic acids and many other equally local events, the control of which is still very much a mystery. A local vasodilatation (p. 63) increasing the blood supply, plays some part.

### 8.3. Neoplastic Growth

Neoplasms are a pathological group of tissues in which growth has been studied with considerable profit. In this case there has been great incentive to research since neoplastic growth is a major human disease. Neoplasms are relatively dedifferentiated tissues with an abnormally high and uncontrolled rate of growth and proliferation. They develop with alarming frequency in man, and rather frequently in certain genetic strains of domestic and laboratory animals. In the laboratory it is now possible to induce them at will, with a number of agents, so that they are being studied extensively *in situ*, *in vitro*, and as transplants. They have clearly great biological, as well as clinical, interest (Huxley, 1958).

The incidence of spontaneous and naturally acquired neoplasms is directly related to the degree of differentiation of the animal, so that they are more common in adults than in young individuals and in insects and the higher vertebrates than in the "lower" animals (Scharrer and Lochhead, 1950). In general the growth rate of normal tissues is inversely proportional to their degree of differentiation, and among mice it is those with a low normal rate of growth which most frequently incur neoplasms (Robertson, 1923, p. 272). This seems paradoxical: a neoplasm is likely to be more conspicuous in a slowly growing animal, of course, but the paradox appears to be genuine, even so.

Since the potential growth rate of most cells is very high (p. 11), a low

normal rate *in vivo* indicates relatively strong systemic inhibition (p. 89) and consequently the frequency of uncontrolled neoplastic growth appears to be proportional to the strength of the normal inhibition. This has given rise to one of the main theories of the cause of neoplasia, the escape theory, which further postulates that inhibition may actually induce a proportionate tendency or power to escape. In fact, carcinogens appear to do just this: they inhibit growth (p. 106) and they stimulate escape. If the tendency to escape were no more than simply spontaneous, it should yield results most frequently in the least inhibited, but fortunately neoplasms are quite rare in young children. When they do occur in children, however, they are particularly malignant, that is rapidly growing (Saxton 1952), presumably because the systemic inhibitor itself is still very weak. The malignancy of teratomata, or neoplasms of the gonads (J. Needham 1942; Berrill, 1943) is probably explicable in the same way. In fact, the power to escape must be something more than a simple effect of the intensity of the systemic inhibitor since the latter is continuing to act in the body at large throughout carcinogenesis and subsequently.

Neoplasms display a range of grades from simple, localized, *benign tumours* to very malignant invasive *cancers*. The range is one of increasing growth rate, with which is correlated a proportionate decrease in differentiation and an increase in locomotor mobility. These correlations are similar to those in cultures of normal cells *in vitro* (p. 89) and, in fact, cultured cells very readily become neoplastic (p. 100).

A high growth rate makes extra demands on the material reserves of the body and some cancer patients are emaciated in consequence, but in most cases the absolute growth rate is not remarkably high (Medawar, 1942), and secondary effects, for instance the encroachment on healthy tissues and organs, causing stress due to pain, are sometimes more serious factors in causing emaciation. Locomotor mobility is shown in the phenomenon of *metastasis*, the property of emigrating as detached cells or small groups of cells which pass via the circulation to other loci; they proliferate here also and encroach upon other tissues, starving them and disturbing their function. Whereas normal cells halt when they come in contact with others (p. 91), cancer cells no longer do this (Abercrombie and Heaysman, 1954).

Virtually all organs are liable to cancer, which is known as a *carcinoma* in epithelial tissues, a *sarcoma* in mechanocytes and a *leukaemia* in blood tissues (Fig. 8.1). *Ascites* is a type originating from a fixed tissue, but secondarily developing the power of proliferating as a culture of free cells, in the lymph and the other body fluids. Tumours of glands are called *adenomas*, those of the liver *hepatomas*, and so on. The term *tumour* is often used synonymously with neoplasm and a *cancer* is usually taken to mean a malignant neoplasm (Huxley, 1956).

Cancerous neoplasms differ from normal tissues also in their transplantability and transmissibility: some will grow if grafted into another individual of the same species, or even of another species. This is related to the fact that they do

not evoke an immune reaction so strongly as normal tissues do when so grafted. Like embryonic tissues, therefore, they have a low immunological specificity, as well as the low grade of differentiation and the high growth rate. This is the main line of evidence in favour of a second main theory of cancer, that it is a return of the tissues to an embryonic condition. In fact embryonic tissues also have a particularly high resistance to any inhibition of their growth (Medawar, 1940) so that this is consistent with the first theory, that the cells escape from inhibition.

The differences between neoplastic and normal tissues have been investigated very extensively, since this is an essential preliminary to the practical aim of curing or preventing cancer, and already this has greatly extended the analysis of the growth process. Many differences have been found, but the important point is that most have proved to be merely quantitatively, and rather slightly, different from normal. One of the more impressive differences is that neoplasms are more resistant to drugs and other agents than normal cells (G. and E. Klein, 1957). Other gross differences are that, in addition to their reduced differentiation, they show a more chaotic texture, a high cell density, and numerous degenerating cells. The latter are attributed to the usually deficient vascularization and therefore to an inadequate supply of oxygen or of other nutrients; nevertheless they may promote the growth of the remainder (Glucksman, 1951) by their autolytic products.

At the cell level the main differences from normal are that both cell and nucleus are very variable, but usually large in size. The nucleoli are large, often complex or multiple and the chromosomes are often polyploid or polytene. The chromosomes also seem to become much stickier than normal (Koller, 1943). The mitochondria are very variable in size and number but are usually fewer than normal (Oberling and Bernard, 1961; Le Breton and Moulé, 1961). The cytoplasm is usually denser (Ludford, 1951) because the ergastoplasm is in the form of discrete microsomes typical of rapidly dividing cells (Fig. 11.2), rather than in the form of a lamellar endoplasmic reticulum (Hagenau, 1958; Mercer, 1959; Slatterback and Fawcett, 1959). The total amount of the ergastoplasm is very variable and may be subnormal (Oberling and Bernhard l.c.; Le Breton and Moulé, l.c.). Both mitochondria and microsomes contain a subnormal amount of protein, while the ground cytoplasm and nucleus have an excess (Laird and Barton, 1956). The Golgi body becomes embryonic in type (Oberling and Bernhard, l.c.). Caspersson and Santesson (1942) have recognized two types of cell: *A* cells or stem cells, rapidly dividing, and rich in ergastoplasm and heterochromatin, and *B* cells or somatic cells, slowly proliferating, but also with basophilic cytoplasm and large nucleolus. However, both types occur in some normal tissues, also, and moreover, even the somatic type has the potential characteristics of a proliferating cell (p. 188).

At the molecular level many of the enzymes associated with the functions of a normal differentiated cell are absent, or deficient (Greenstein, 1954; Le Breton and Moulé, 1961). At one time it was believed that, in fact, there

was a deficit in all enzymes concerned with aerobic respiration in the Krebs cycle (p. 258) but this is now questioned (Le Breton and Moulé, *i.c.*). On the other hand, Warburg's classical discovery that there is a high rate of glycolysis (p. 258) does seem to be confirmed, notwithstanding some severe criticism (Hieger, 1961). The glycolysis, because it is so rapid, continues in the presence of abundant oxygen, although the Pasteur effect is still in operation, as in normal cells.

There is a deficiency of most of the B-vitamins (p. 303) in neoplasms; this is to be expected since these vitamins are the coenzymes of a number of the enzymes which disappear (Griffin, 1960). An exception is biotin, which, significantly enough, also is carcinogenic, in supernormal concentrations. There is a deficiency also of the fat soluble vitamins, A and E.

Some enzymes are more active than normal and these are mainly enzymes concerned with biosynthesis, directly or indirectly. Those concerned indirectly, that is in breaking down materials for subsequent rebuilding (p. 335) include cathepsins (Lehmann, 1959), nucleic acid depolymerases and phosphatases. The enzymes of the gluconic acid shunt (p. 259) also are more active than normal and may help in the synthesis of nucleic acids (p. 220) by degrading glucose to pentose sugar (Sahasrabudhe, 1958). Tumours resemble embryonic tissue in having a high activity in this pathway and in the main glycolytic pathway. It may ultimately be possible to attack cancer by the use of analogues of key metabolites of these pathways, particularly the gluconic shunt, since normal tissues should be much less disturbed by blocking it.

There is a shift from using carbamyl phosphate mainly for the synthesis of urea for excretion, to using it in the synthesis of pyrimidine bases for the nucleic acids (Glass, 1958). The carcinogenic action of urethane and other carbamic esters may be relevant here. Both types of nucleic acid are synthesized more abundantly than normal (Le Page and Heidelberger, 1950) though the amount per cell is average, or even subnormal (Le Breton and Moulé, 1961).

The rate of protein synthesis compared with that of normal cells is even more rapid: amino acids (*a.a.s.*) may be incorporated six times as rapidly as normal (Zamecnik *et al.*, 1948). The rate is not so fast in some tumours, however (Brachet, 1957), and there is less than normal of some proteins, for instance those capable of acting as antigens in other animals and also the histones and other basic proteins (Hieger, 1961). The latter are mainly associated with nucleic acids in normal cells and tend to stabilize them, perhaps also to restrain their activities. Most other protein fractions are higher than normal. As in embryonic tissues the non-essential amino acids (p. 288) are more abundant than in normal adult tissues and the essential *a.a.s.* less so; the deficiency of tryptophan is particularly marked (Birks, 1961). The essential *a.a.s.* are more concerned with differentiation and the non-essential ones with growth.

The concentration of lipids is higher than normal (Luck, 1949), particularly cholesterol and the phospholipids. One P-lipid, which has been called "maligno-lipid" but not yet identified, may be peculiar to cancerous tissue (Le Breton and

Moulé, l.c.). If so it is probably the only substance yet found to be absolutely peculiar to these tissues.

The glucose content is subnormal probably because glycolysis uses it so wastefully: for the same reason there is more of lactic and pyruvic acids than normal. Organic phosphate is in supernormal amount while the amount of inorganic phosphate ( $P_i$ ) may be as low as 20 per cent of the normal. There is a remarkable excess of iron in many tumours, as much as five times the normal amount, whereas calcium is only 50 per cent and potassium as low as 16 per cent of the normal concentration. The water content is high (Senftle and Thorpe, 1961), again as in embryonic tissue.

Tumours cause a number of systemic changes, in addition to the direct effects of their errant behaviour. They use more of the plasma proteins, particularly the  $\alpha_2$ -globulin fraction (Kent and Gay, 1960); the ratio of globulin to albumin in the plasma increases, (Luck, 1949), perhaps to meet this demand. There is a very significant increase in a particular plasma  $\alpha$ -globulin (Darcy, 1957, 1960). This is not the same as *fetuin*, another protein which increases under conditions of increased cell-proliferation (Puck, 1959). The enhanced catheptic activity (Lchmann, 1959) actually extends to the body in general and the consequent mobilization of protein largely accounts for the emaciation noted earlier (p. 96). There are changes in the blood cells and in their processes of formation in the bone marrow. Other systemic effects have been discovered, some affecting particular organs, especially the liver. Most of these changes are no doubt secondary and probably none is an actual cause of cancer.

Metastases of course can affect many parts of the body. In addition, the centre of tumours tends to become necrotic, probably owing to the inadequate blood supply, which is rather characteristic. Toxic products are therefore released and no doubt affect the whole body.

These are the main symptoms of the typical neoplasm and they are sufficiently consistent to encourage the belief that the proximate cause may be the same in all cases, notwithstanding the variety of agents which can induce tumours. The proximate cause remains very obscure but it seems almost certain that there must be eventually some kind of somatic mutation, that is to say a relatively permanent change in one or a restricted group of cells which then continue to "breed true" as they multiply. There are initial difficulties in accepting this explanation but they are not insuperable. In the first place the rate of incidence even of spontaneous tumours, is higher than orthodox mutation rates in the germ line. However, most somatic tissues are much more exposed to mutagenic environmental hazards than the germ line and, in addition, there is a spontaneous tendency for the chromosome number to become very variable in somatic tissues. In somatic cell lines even changes acquired purely by the cytoplasm could be permanently self-perpetuating.

Another difficulty is that the neoplastic changes affect almost every component and property of the cell to some degree, rather than a single component to the dramatic extent normally associated with mutation. Even this is not an

insuperable objection, if the mutation does, in fact, involve one or more whole chromosomes. The number of different chromosomes in the genome is never very large so that there is a relatively high probability of the same change recurring fairly frequently, by chance alone, quite apart from the probability that, in fact, many mutagenic agents are not fortuitously distributed. Hyperploidy of certain of the chromosomes is perhaps more likely to produce the necessary state than a hypoploid condition, and it may be significant that the average chromosome number in cancerous tissue is greater than normal (Oberling and Bernhard, 1961).

An important feature of carcinogenesis which may help to explain the great number of the ultimate changes is that new cancers do not develop immediately, but only after a considerable latent period. This is much longer than most lag periods already encountered and is also a factor which in the past greatly obscured the aetiology of many types of cancer. During the latent period a series of changes may be occurring and it has been suggested (Huxley, 1956) that there is, in fact, a series of mutations, possibly small individually but collectively large. In some cells, it may be supposed, the final result is the complete constellation of changes necessary for active neoplasia.

Mutations in the germ line do not involve such sequences but there the number of cell generations in any individual animal is small. Where there is scope for these serial changes, in some somatic lines, there is the further possibility that each predisposes the cell to undergo further changes and that these are all in a specific direction. The only basic requirement is that the normal rate of cell proliferation shall be high enough to provide the opportunity for serial mutational change. In many somatic tissues the normal rate is probably high enough to explain the incidence of spontaneous cancers, and mutagenic carcinogens will further increase the incidence. The culture of cells *in vitro* has shown that provided the rate of proliferation is adequate a number of neoplastic lines of cells inevitably appear (Hieger, 1961). Cells *in vitro* have been shown to undergo spontaneous somatic changes rather frequently (Puck, 1957). We can suppose that under normal conditions *in vivo* the chances of a serious outbreak of tumours before the end of the effective reproductive period (p. 27) are too small to have been subjected to more intense natural counter-selection and that cancer is, in fact, one of the fortuitously acquired disabilities of the elderly.

There is definite evidence that the final, permanently neoplastic state is developing gradually during the latent period. In some induced neoplasms the changes are at first fully reversible; the growth can be induced to regress and then can be stimulated to progress once more. In many respects the tissues remain labile until the final irreversible stage (Quastler and Baer, 1948). This lability seems quite foreign to orthodox mutational changes in the germ line, however small, but in somatic tissues the true or permanent mutational change may occur late in the postulated sequence of changes. It is probably also important that the significant changes occur only in a very small percentage of cells

of the somatic tissue in question whereas most of the properties which can be measured are those of the more heterogeneous population of cells as a whole. Nascent neoplasms themselves continue to produce a considerable percentage of postmitotic cells, that is cells destined never to divide again, just as normal somatic tissues do.

Once a cell has appeared with a growth and proliferation rate above normal its progeny will increase as a percentage of the total and the properties of the whole will become progressively more uniform and more completely those of a permanent neoplasm. The significant change is probably irreversible from the moment it occurs in the cells actually involved, and in their progeny. In a population of cells any change other than the neoplastic, occurring in a small group would remain undetected, since the progeny of the group would not increase differentially. It is important to note that a neoplastic line automatically acquires the advantage of an expanding population, in which there could occur a proportionate number of mutations to even higher rates of proliferation. It has been observed that individual tumours do pass through the whole gamut of grades of malignancy to their final state.

While there may well be this long series of changes the results of some of the work indicate an initial critical one known as *induction*, and subsequent minor changes called *promotion* (Huxley, 1957). In the case of acquired neoplasms the first change is due to a primary carcinogen, or cancer-inducing agent, whereas promotion may be effected by otherwise innocuous cocarcinogens. Because of the properties of an exponential curve of multiplication the later promotion stages may appear the more dramatic and important. Laird and Barton (1959), inducing cancer in the rat's liver by a chemical carcinogen, observed the usual latent period and then a sudden increase of 50 per cent in the number of liver cells within a particular period of two days. The increase, however, was due to the progeny of a very few cells initially induced, not more than  $10^{-7}$  of the total, multiplying undetected for a considerable time. In fact, in this case there may have been no real promotor—except time! On the other hand, a mathematical treatment by Burch (1960) of experimental results indicated a process with two distinct stages, no more and no less. Whether these represent induction and promotion respectively is open to question: from the other evidence it does not seem that promotion is a definite, unique event.

It seems necessary at this point to emphasize that there are some cases of hyperplasia which resemble neoplasms but are not due to local somatic mutation. In these cases the replacement rate increases slightly in all the relevant cells of an organ, owing to a systemic factor acting on them all. Such an increase in a whole population need not be very great to become rapidly serious; examples include an hyperplasia of the pituitary gland, when the secretions of the ovary are prevented from exerting their normal negative feedback action on the organ (Berenblum, 1950), and of the thyroid under the stress of low iodine concentration in the body. Some oncologists have recognized the rather special status of this type of hyperplasia, whereas others (Hieger, 1961) believe

that it is the main type. However, it is rarely malignant or metastasing, at least as a primary phenomenon (Kirschbaum, 1960) and is often regarded as a simple physiological hyperplasia.

If, as seems likely, the neoplastic change is intracellular, then, strictly speaking, neoplasms are growth phenomena determined below the tissue level, though soon manifest at the higher level. Moreover, unless the relevant mutations are *directed*, unless there is a specific causal relationship between the inducing agent and the resulting growth properties of the mutant (p. 109), we may learn little about growth at any level purely from the study of carcinogens. In fact, a very large number of very varied agents have been found carcinogenic (Table 8.1) and this might imply that they are indeed acting non-specifically (Hieger, 1961), merely triggering off an autogenous process, just as cell division (p. 338), the fertilization of the egg (p. 425), and nerve and muscle activities are triggered. A number of carcinogens in fact do also trigger other processes on this list. On the other hand some certainly have a direct effect on normal growth and metabolism (Birks, 1961), and there are certain classes of rather specific and potent carcinogens which merit further consideration.

Some neoplasms are spontaneous in the sense of having no detected external cause, or of recurring in strains or species with a genetic predisposition to develop them. Thus, human retinoblastoma is a spontaneous neoplasm, depending on a single Mendelian factor pair. However, even supposedly spontaneous tumours probably require also a more proximate cause (Kirschbaum, 1960). The mammary carcinoma of mice is not a purely spontaneous inheritance in susceptible strains, as once believed, but is due to subcellular particles transmitted to the young in the mother's milk. Intracellular particles are now known to be the cause of a number of natural neoplasms and similar agents may be inferred for others. They are self-reproducing bodies so that their entry into normal cells might be said to constitute a cytoplasmic mutation (p. 99). The agent transmitting mouse mammary carcinoma is a cytoplasmic nucleoprotein particle of the size of the ribosomes (p. 146), 20 to 30 m $\mu$  in diameter, while that of the Shope rabbit papilloma is 65.6 m $\mu$ ; the Rous chick sarcoma agent is a phospholipid-ribonucleoprotein complex, 70 m $\mu$  in diameter and that of avian leucocytosis is a particle of 120 m $\mu$ .

Not all of these, as found in the eventual neoplasm, were necessarily the initial causal agents of their respective neoplasms. In other cases induction is associated, directly or indirectly, with infection by foreign organisms—viruses, bacteria, or larger parasites such as *Monocystis*. Since virtually all known carcinogens are extraneous, i.e. not normal metabolites, there is always the problem of finding out how direct their action is, on the cells which eventually respond. Mechanical irritation, if it is ever truly carcinogenic (Berenblum, 1950), may operate by eventually introducing foreign bodies, but, on the other hand, it may increase the chance of a neoplastic mutation, through stimulating reparative cell proliferation.

It seems likely that some, at least, of the reputed carcinogens (Table 8.1) may

TABLE 8.1

**Reported Carcinogenic and Anticarcinogenic Agents****1. Carcinogenic agents****1.1. Physical factors**

? Mechanical irritation . . . . .	{ Berenblum, 1950 Black, 1946 Pullinger, 1944 Hieger, 1961 [J. Needham, 1942 Mottram, 1942 Ludford, 1951 Hollaender, 1956
Bakelite, metal foil . . . . .	
High temperature } . . . . .	
Low temperature } . . . . .	
Ultra-violet rays . . . . .	{ Berrill, 1943 Saxton, 1952 Spear, 1953 Burch, 1960
Ionizing radiations . . . . .	{ Berrill, 1943 Saxton, 1952 Spear, 1953 Burch, 1960

**1.2. Chemical agents****1.2.1. Inorganic**

HCl, NaCl, selenium compounds, arsenious acid . . . . .	Cook, 1943
Asbestos . . . . .	Hieger, 1961
Thorotrast ( $\text{ThO}_2$ ) . . . . .	Heilbrunn, 1956
Chromates . . . . .	Hieger, 1961
Cobalt salts} . . . . .	Heath, 1954
Nickel salts} . . . . .	Heilbrunn, 1956
Beryllium salts . . . . .	

**1.2.2. Organic****1.2.2.1. Fat-soluble**

Chloroform, carbon tetrachloride . . . . .	Heilbrunn, 1956
Aniline . . . . .	Ludford, 1951
Polycyclic hydrocarbons, particularly benzpyrenes, benzanthenes, cholanthenes, and the nitrogen-containing dibenzacridines, dibenzcarbazoles, azobenzenes, azotoluenes, naphthylamines . . . . .	{ Badger <i>et al.</i> , 1942 Cook, 1943 Heilbrunn, 1956
Thiocresol . . . . .	
Nitrogen mustards . . . . .	
Cotton seed oil . . . . .	Scharrer and Lochhead, 1950
Croton oil . . . . .	{ Boyland, 1949 Dodds, 1949
Oil of camphor } . . . . .	{ Peacock and Beck, 1948 Shubik <i>et al.</i> , 1953
Oil of eucalyptus} . . . . .	
Sesame oil . . . . .	{ Setala, 1954 Heilbrunn, 1956
Oil Orange TX . . . . .	{ Rawles, 1948 Bonzer <i>et al.</i> , 1954

**1.2.2.1.1. Lipids with metabolic significance**

Glycerides (dietary, as vehicles) . . . . .	{ Tannenbaum, 1942 Webster, 1942
Sterols (oestrone, testosterone) . . . . .	{ Saxton, 1952 Huxley, 1957
Aminostilbene (sterol analogue) . . . . .	{ Klein and Klein, 1957 Haddow, 1948
Cholesterol . . . . .	{ Hieger, 1957 Nicol and Snell, 1954
Cortisone . . . . .	{ Baruah, 1958

TABLE 8.1—(cont.)

1.2.2.2.2.	<i>Water-soluble</i>		
	Trypan blue . . . . .	Iwase and Fujita, 1955	
	Other dyes . . . . .	Ludford, 1951	
	Urethane . . . . .	{Noble and Millar, 1948 Boyland and Koller, 1954	
	Acetylaminofluorene . . . . .	Laird and Barton, 1959	
	Tannic acid . . . . .	Heilbrunn, 1956	
1.2.2.2.1.	<i>Normal metabolites and related substances</i>		
	Glucose . . . . .	{Badger <i>et al.</i> , 1942 Mottram, 1942	
	Cystine . . . . .	Kirschbaum, 1960	
	Cysteine . . . . .	Webster, 1942	
	Casein . . . . .	Tannenbaum and Silverstone, 1949	
	Adenine, guanine, cytosine . . . . .	Parsons <i>et al.</i> , 1947	
	Folic acid (pteroylglutamic acid) . . . . .	Boyland, 1949	
	Xanthopterin (on kidney) . . . . .	Haddow, 1948	
	Biotin . . . . .	Briggs, 1959	
	Xanthine oxidase . . . . .	Haddow <i>et al.</i> , 1953	
	APGH . . . . .	{Li, 1950 Hemingway, 1960	
1.2.2.3.	<i>Macromolecular substances and living organisms</i>		
	Mouse milk factor . . . . .	Passey <i>et al.</i> , 1950	
	Roux chick sarcoma extracts . . . . .	Claude, 1935-9	
	Virus-like, filter-passing particles from existing tumours . . . . .	{Huxley, 1957 Mellors, 1958 Haddow, 1948	
	Polyhedral virus of sawfly . . . . .	Bird, 1949	
	Aureogenus virus of plants (e.g. sweet clover) . . . . .	Black, 1946	
	Bacteria (in <i>Nereis</i> , and for crown gall of plants) . . . . .	Scharrer and Lochhead, 1950	
	Actinomycosis, in cattle . . . . .	Huxley, 1956	
	<i>Monocystis</i> , in <i>Ciona</i> . . . . .	Scharrer and Lochhead, 1950	
	Nematodes and trematodes . . . . .	Huxley, 1957	
1.3.	<i>Physiological and pathological agents and conditions</i>		
	Nerve-section ( <i>Leucophaea</i> ) . . . . .	Scharrer and Lochhead, 1950	
	Ablation of corpus allatum . . . . .	Scharrer and Lochhead, 1950	
	Other tumours . . . . .	Russ and Scott, 1942	
	Overripe eggs (frog, <i>Nereis</i> ) . . . . .	J. Needham, 1942, p. 260	
	'Necrosin' . . . . .	Menkin, 1949	
	Thyroid deficiency (fishes) . . . . .	Huxley, 1957	
	Absence of dermal papilla (on hair follicle) . . . . .	Wolback, 1950	
	Hybrid condition (genic imbalance) . . . . .	{Rhoads, 1949 (Berg and Gordon, 1953	
	Emotional stress . . . . .	{Naylor, 1961 (Lang Stevenson, 1961	
2.	<i>Anticarcinogens</i>		
2.1.	<i>Physical factors</i>		
	Light . . . . .	{Morton <i>et al.</i> , 1940 (Apperly and Cary, 1942	
	Ionizing radiations, low dosage . . . . .	Apperly and Cary, 1942	

TABLE 8.1—(cont.)

2.2. <i>Chemical agents</i>					
2.2.1. <i>Inorganic</i>					
Acidity ( $\text{NH}_4\text{Cl}$ ) . . . . .	Thompson <i>et al.</i> , 1943				
$\text{H}_2\text{O}_2$ . . . . .	{ Holman, 1957 Sugiura, 1958 }				
2.2.2. <i>Organic</i>					
Quinones }	Brachet, 1957				
Dinitrophenol)	{ Scharrer and Lochhead, 1950				
Bromobenzene . . . . .	{ Crabtree, 1945 Boyland, 1949 }				
Unsaturated dibasic acids . . . . .	Crabtree, 1945				
Stilbamidine, chloroethylamine . . . . .	Boyland, 1949				
Nitrogen mustards in low concentration . . . . .	{ J. Needham, 1942 Dodds, 1949 }				
Reserpine, chlorpromazine (tranquillizers) . . . . .	Boyland, 1949				
Urethane . . . . .	Belkin and Hardy, 1957				
Trypan blue . . . . .	Florijn and Smits, 1949				
Iwase and Fujita, 1955					
2.2.2.1. <i>Metabolites, analogues and related substances</i>					
Body-fat . . . . .	Cook, 1943				
Vitamin E . . . . .	J. Needham, 1942, p. 265				
Glucose . . . . .	Sahasrabudhe, 1958				
Maleic acid . . . . .	Crabtree, 1945				
Heparin, Shear's polysaccharide . . . . .	Heilbrunn, 1956				
Histone and other proteins . . . . .	{ Boyland, 1949 Brachet, 1957 }				
Methionine . . . . .	Kirschbaum, 1960				
Amino acid analogues . . . . .	Brachet, 1957				
N-dichloroacetyl-DL-serine . . . . .	Levi <i>et al.</i> , 1960				
Adenine, guanine . . . . .	Parsons <i>et al.</i> , 1947				
Guanine analogues . . . . .	Kidder <i>et al.</i> , 1949				
Uracil analogues . . . . .	Brachet, 1957				
Ribonuclease . . . . .	{ Ledoux, 1955 Brachet, 1957 }				
B-vitamins . . . . .	Rhoads and Kensler, 1941				
Folic acid analogues, pterins . . . . .	{ de Ropp, 1949 Sahasrabudhe, 1958 }				
p-Aminobenzoic acid . . . . .	Boyland, 1949				
Riboflavin . . . . .	Miller <i>et al.</i> , 1948				
Choline . . . . .	Engel <i>et al.</i> , 1947				
Pantothenic acid . . . . .	Lewisohn <i>et al.</i> , 1941				
Thyroxin . . . . .	Huxley, 1957				
Adrenal cortical hormones . . . . .	Hemingway, 1960				
2.3. <i>Physiological agents</i>					
Reduced caloric intake . . . . .	Potter, 1945				
Reduced B-vitamin intake . . . . .	Jones, 1942				
Mitomycin C (antibiotic) . . . . .	Sokoloff <i>et al.</i> , 1959				
Antigens . . . . .	Michael and Emde, 1940, 1942				

act indirectly, and others, as suggested above, simply as non-specific promoters (Huxley, 1957) of a neoplasm already induced by one of a more limited number of specific agents. The effects of two carcinogens often summate, and sometimes they compete; the latter in particular implies that they are acting at a

common site. There are two outstanding groups of extrinsic carcinogens, ionizing radiations and polycyclic hydrocarbons; it is significant that both have also mutagenic properties, with powerful effects on the chromosomes of germ cells. Paradoxically, both also inhibit normal growth. A number of mutagens outside the class of polycyclic hydrocarbons also are carcinogenic, and by no means all of these hydrocarbons are, so that the oncological classification does not correspond to any generic chemical classification, which depends on the backbone or nucleus of the molecule rather than on its specific active groups.

It has been possible to recognize the following structural and other properties common to many carcinogens of whatever chemical class: low water-solubility, a ring structure, double bonds between some of the carbon atoms, with the *trans* configuration about the plane of these bonds, and a high  $\pi$ -electron density in a particular meso-region of the molecule (Daudel, 1946; Coulson, 1953). These properties in general confer a high power to activate other molecules, and this activation might be the direct cause of mutation. The double bond in their molecules appears to confer growth-promoting properties on the unsaturated fats (p. 298) and other substances and there are other examples of its possible significance for growth. Fluorescence also is a common property of this kind of activating molecule and many carcinogens in fact are fluorescent. Birks (1961) points out that tryptophan, through the first excited  $\pi$ -singlet state of its molecule, emits strongly in the region absorbed by many carcinogens, and that the cancerous change in a tissue leads to marked depletion of tryptophan-containing proteins. The purines emit weakly in the same region.

The action of ionizing radiations on the nucleus involves oxidation (Collinson *et al.*, 1950; Wood, 1959) and physiological antioxidants such as reduced glutathione (GSH) give a degree of protection. Some carcinogens are found to inhibit the reducing actions of GSH and ascorbic acid (Boyland, 1949) and probably combine with the SH groups of proteins. The changes in respiratory metabolism when tissues become neoplastic (p. 98) therefore may be due to an effect of the carcinogen on the oxidation-reduction systems of the cytoplasm, whether directly or via the nucleus. Lovelock *et al.* (1962) believe that all carcinogens are electron acceptors and therefore oxidizing agents, and their view seems to fit the main body of fact better than earlier attempts to show that they were, in fact, electron donors. They may be both (Allison and Nash, 1963) and this may be the main reason why some of the information about oxidation-reduction changes seem paradoxical. This may also be due partly to the usual difficulty (p. 412) in distinguishing between the primary, direct effects of an external agent and the positive responses it induces in the organism—often counteracting the primary effect (p. 423). For instance, Fiala (1958) found an increase in concentration of free SH-groups in the early stages of carcinogenesis followed later by a deficit. The antioxidants, vitamins A and E, also become depleted (Sobel and Marmorston, 1954); they are also anticarcinogenic and so conceivably may become exhausted, along with SH-compounds and ascorbic acid, in an initial reaction with an oxidizing carcinogen.

It may seem paradoxical that an oxidizing agent should provoke tumours with a predominantly glycolytic type of cellular respiration which is potentially, at least, anaerobic. However, if carcinogens should behave like dinitrophenol (DNP) and other substances (p. 375), that is, by uncoupling the aerobic, terminal stages of oxidation from the necessary reactions of phosphorylation, then a switch to the alternative pathway of glycolysis, as in the normal Pasteur effect, might be the only means of survival. Once committed to glycolysis the cells perhaps automatically become progressively more embryonic in metabolic type. The Krebs cycle now may be used for synthesis rather than for respiratory catabolism (p. 237), and so the whole balance of metabolism may be shifted in favour of anabolism. The initial change might, in fact, correspond to induction and the subsequent ones to promotion.

This response of the respiratory system might well explain another paradox which is rather striking and puzzling, namely that most carcinogens are found to inhibit growth, including that of existing tumours! The respiratory adjustment may be the critical step enabling the cells to escape from this inhibition. In the arrest of growth during diapause in insects an inactivation of the terminal oxidases, in fact, plays a critical part (p. 426).

Until recently it would not have seemed likely that lipid-soluble substances could significantly affect normal oxidation-reduction processes in the body but it now seems probable that a whole series of the steps in the normal electron-transfer system of cell respiration occurs effectively in a lipid medium (Green, 1959). Moreover, the steroids, which have considerable chemical affinities with many of the hydrocarbon carcinogens, appear to be active physiological *redox* agents (Villee, 1962). The steroids of the adrenal cortex are there associated with high concentrations of two further physiological antioxidants, ascorbic acid (vitamin C) and reduced glutathione (GSH). The main redox action of the steroid hormones is connected with the pyridine co-dehydrogenases (p. 308). Burzatta (1961) has made a detailed assessment of the role of the steroids in carcinogenesis, particularly in this redox role. Some of the physiological steroids, and others, themselves cause neoplasms: the cholic acid of the bile is readily converted to a rather potent carcinogen. Overripe unfertilized eggs tend to become teratomatous (Witschi, 1930), perhaps owing to changes in their steroids, which appear also to play a significant part in normal morphogenesis (J. Needham, 1942; Burzatta, 1961) as well as in normal growth (p. 370). Hemingway (1960) believes that in neoplasms, as well as in regenerating tissues, a normal inhibitory control of growth by the adrenal corticoids is in some way neutralized, locally (p. 428).

No doubt there is still further significance in the fact that most carcinogens have a high solubility in lipid solvents. The property enables them to penetrate the lipid component of the cell membrane. Even a simple lipid such as croton oil, itself innocuous, promotes the action of primary carcinogens, probably by acting as a vehicle for them (Setala, 1954); it is one of the best known co-carcinogens. The number of potent primary carcinogens is probably limited

by the dual requirement for lipid-solubility and oxidizing power. Apparently croton oil itself is not without effect on terminal oxidation (Allison and Lightbown, 1961) and it potentiates the action of ionizing radiations (Shubik *et al.*, 1953) so that its promotion of carcinogenesis may depend on something more positive than its vehicular action.

Setala (l.c.) suggests that the polar-non-polar structure of the molecule may be the really important property of croton oil and certain other lipids. They may promote carcinogenesis because of their solubility in both lipid and aqueous media. The surface-active *Tweens*, *Spans*, detergents and bile acids also behave in this way, as co-carcinogens; apocholic acid, the carcinogenic derivative of the bile acids (p. 240) can act as its own vehicle. There are other primary carcinogens with polar groups, oxygenous or nitrogenous, although some of the most potent certainly are pure hydrocarbons and perhaps always need a vehicle. The carcinogenic properties of a molecule are often greatly affected by the addition or subtraction of methyl groups, which change the ratio of polar to non-polar components of the molecule.

These activities, respiration, cell permeation, etc., concern the general running of the cell, and therefore its cytoplasm rather than its nucleus. Consequently a critical question at this point is whether the effect of carcinogens on such activities can be mediated through the nuclear changes discussed earlier. The effects on respiration seem to be common to tumours in general and the essential nuclear change is thought to be equally generic. Reasons have been given (p. 99) for accepting the possibility that somatic mutations could be extensive enough to explain why there are changes in almost every activity of the cell as it becomes neoplastic.

On the other hand, some of the evidence from chemical carcinogens seems to indicate that there is a direct effect on the cytoplasm, perhaps in parallel with the effect on the nucleus, which seems equally direct on the evidence from ionizing radiations and other mutagenic carcinogens. Carcinogens must pass through the cytoplasm on their way to the nucleus and chemical carcinogens are found to accumulate on the cytoplasmic particles (Boyland, 1948*b*; Waddington and Goodhart, 1949). Viruses and other particulate carcinogens in general invade the cytoplasm only and there is no good evidence that they affect the nucleus. However, some of them evoke tumours without any lag period, so that they appear to short-circuit the more usual path of carcinogenesis and perhaps transmit neoplasia rather than induce it *de novo*. This difference may explain the contention of Hieger (1961) that some tumours (in addition to the parapathological adenomas of the endocrine organs) arise throughout a tissue and not only in a few sharply localized cells. The latter mode, which is generally thought to be the most common (Laird and Barton, 1959) must almost certainly be associated with chromosomal mutation. Primary carcinogenesis, therefore, may always involve mutation but it is not yet clear if the cytoplasmic changes are all consequential to this: they might be parallel effects.

It is noteworthy that the main effect of ionizing radiations is oxidative and

that deoxyribonucleic acid, DNA, the sensitive form of nucleic acid, and usually regarded as the genetic "information" of the nucleus, has its ribose sugar in a more reduced form than ribonucleic acid, RNA, which is more insensitive to irradiation. Oxidation-reduction changes therefore predominate in both nucleus and cytoplasm and the question whether these are in parallel or consequential is important. If the cytoplasmic changes are due secondarily to the mutational changes in the nucleus then these have the quality of directed mutations (p. 102), that is to say mutations functionally related to the nature of the mutagenic agent. In the present instance oxidizing agents cause mutations which change the oxidation-reduction properties of the cytoplasm. As yet few directed mutations have ever been described and they are not thought to be at all usual, so that the present case should be investigated further. If the effects on nucleus and cytoplasm are not consequential but in parallel then there is the further problem whether cancer can sometimes result from the one alone, and if so whether the changes in the other need ever appear.

This would imply more than one pathway of carcinogenesis and perhaps more than one type of neoplasm. In the preceding discussion the generic nature of both has been stressed but this was mainly in order to simplify the initial approach, and we may have to envisage the possibility that there are a number of paths and types. Certainly there is some justification for applying Occam's principle: virtually all tissues are subject to cancer (Huxley, 1958) and when the neoplasm develops it has little immunological specificity, and much the same properties, whatever its origin. On the other hand, there are some specific differences between tumours and between modes of induction, and these deserve more attention.

A number of chemical carcinogens have been found specific to particular tissues. In many cases this may be simply because they accumulate there: for instance, butter yellow, *o*-azoaminotoluene, probably accumulates in the liver for metabolic reasons, xanthopterin in the kidney during excretion, urethane and other air-borne agents in the lungs, and the carcinogens of tar on the skin. Soot may be specific to the scrotum because this is a particularly sensitive area of the skin, in a generic rather than a specific way. These examples include all the best-known cases of apparent restriction of action to particular sites so that the question needs to be investigated very critically.

There are also cases where tumours appear to differ when transmitted or transplanted to another species. The Shope papilloma of the cotton-tail rabbit can be transmitted as free cell "particulates" to the domestic rabbit. In its original host the tumour is benign but in the tame rabbit it becomes malignant and also will no longer transmit as cell particles (J. Needham, 1942; Berrill, 1943). This is reminiscent of the difference between prophage and active bacteriophage (p. 157) and may be a phenomenon of this kind rather than a real difference between the neoplastic tissues of the two hosts. Examination therefore tends to emphasize the paucity of real differences between cancers and between modes of carcinogenesis, and the original main question of the relationship between the

nuclear and cytoplasmic changes during carcinogenesis remains a general problem.

It is not the only uncertainty: knowledge of carcinogenesis has given a superabundance of clues, which may eventually fit a unified theory, but at present it is possible to emphasize particular bodies of results and to develop very different main theses. Recent examples are the neurological theory of Lang Stevenson (1961) and the steroid hormonal theory of Burzatta (1961). Clinical evidence bearing on the neurological theory has been collected by Naylor (1961): the theory deserves special emphasis since it has not previously received much attention. No doubt there are fundamental neurological and hormonal aspects of carcinogenesis. The present problem is to relate them to the other aspects.

The main facts about neoplasms which seem reasonably well established are that they are initially sharply localized nests of cells which have acquired an abnormally high rate of growth and proliferation, with decreased differentiation and antigenic specificity and a metabolism changed towards glycolytic respiration and rapid biosynthesis. The change is relatively standard and not specific to any of the host of carcinogenic agents discovered, though these may perhaps induce the change in a number of different ways. Again various tissues may have a different susceptibility to carcinogenesis but this does not necessarily result in different types of cancer. The one certain exception to the generalization that all neoplasms are alike is the endocrine adenoma, which appears to be due simply to an exaggerated physiological response.

We are impressed and appalled by the frequency of cancer in human populations today but in view of the number of possible hazards and of the number of already known carcinogens (Table 8.1) it would be as reasonable to marvel at the relative rarity of the phenomenon. This is very relevant to the solution of the problems of neoplasia, and indeed of normal growth. As an antidote to any feeling of pessimism about an early solution of the problem of cancer it is important to recognize that its solution may simultaneously solve the wider problem of the mechanism of growth in general.

## CHAPTER 9

### *The Growth of Populations of Microorganisms*

IN some respects a population of microorganisms may be compared with a population of cells in the metazoan body: in the laboratory, under appropriate conditions of nutrition, space, temperature and pH, a population of Protozoa or of other microorganisms increases in number and total mass in the same general way as the population of cells in a young metazoan individual, according to a sigmoid curve of size in time (Hinshelwood, 1946). This proves to be a general rather than a specific criterion since, in fact, populations of metazoan individuals under suitable conditions also multiply in accordance with the same sigmoid curve; this has been shown for man (Pearl, 1946), *Hydra* (Loomis and Lenhoff, 1956) and other animals. However, there are other resemblances between populations of microorganisms and tissue cultures.

If the growth of a culture of microorganisms is measured by the number of cells present then, as in a tissue culture, there is a distinct lag phase (Hinshelwood, 1946; Dean and Hinshelwood, 1959) before numbers begin to increase, though in fact it is a period of intense activity; during the later part of the period the individual cells are already growing rapidly, and their volume may increase as much as eight fold (Hershey, 1939). The true lag period of growth is usually but not always relatively short (Lichstein, 1959); during this time spores are germinating (Bisset, 1950; Halvorson, 1959) and vegetative cells are becoming acclimatized or *conditioned* to the culture medium. At one time it seemed possible (Robertson, 1923) that the organisms produced and secreted some specific autocatalytic substance and that the lag period continued until this reached a threshold concentration in the medium. However, no autocatalyst has been identified (Richards, 1941). The amino acids (Gale, 1953) and other metabolites (Halvorson, l.c.) often secreted into the medium (p. 184) and including some which are species-specific (Lockhart, 1959), contribute to the conditioning but they are not autocatalytic agents in the classical sense, since their effect does not increase indefinitely with concentration. Moreover, although the lag period is usually shortened, in most cases the specific growth rate is not increased by seeding a larger number of organisms into a medium—to share the conditioning processes of secretion, absorption and active metabolism. Cells subcultured when already dividing actively show no lag and this is equally true of metazoan cells *in vitro*. Their behaviour therefore depends largely on their own internal state, but this, in turn, is very sensitive to environmental factors. Some metabolites, for instance a mixture of L-alanine and adenosine, will shorten the true lag period of some bacterial spores to 2–5 minutes; the response is effectively a triggered one (p. 425).

The resting microorganism shows little activity of the enzymes concerned in synthesis (Hinshelwood, 1946; Knaysi, 1951), and activity develops during the lag period; at first this is through the activation of dormant enzymes (Halvorson, 1959) but later there is rapid synthesis of new enzyme protein. The amount of RNA in the cell also increases (Caspersson, 1950) but not that of DNA. There is an increase in permeability of the cell and such surface-active agents as Tween 40 can greatly shorten the lag period. The cell takes up water, salts and other materials. The changes are mainly inside the cell: the medium registers a change in electrical conductivity (Krishnamusti and Kate, 1951) but this may be due largely to a change in electrophoretic charge on the cells (Hiss and Zinsser, 1957). Since the addition to the medium of amino acids and other raw materials shortens the lag period (Morrison and Hinshelwood 1949; Dagley *et al.*, 1949), this may be due, in part at least, to an initial shortage of building materials and other metabolites rather than only of the enzymes to utilize them. However, it is now clear that these metabolites also have an important function as enzymic inducers (p. 208) and alanine shortens the lag period in concentrations too low to serve as an effective substrate material (Halvorson, *I.c.*). Growth inhibitors which act during the lag period are among the most potent of inhibitors, since their effect tends to be all or none; above a critical concentration they prevent any cell reaching the stage of division so that, as measured by cell number, there is no growth. The cells are particularly sensitive to all agents during this period, partly no doubt for this reason, and the sensitivity contrasts with the high resistance of spores and other resting bodies.

Two main stages have been recognized in the lag period (Halvorson, *I.c.*), the initial and often very brief triggering which results in a sudden increase in respiration, disappearance of the strong refringence of spores, increase in stainability and decrease in resistance to deleterious external agents. Spores of all bacteria examined contain between 6 and 12 per cent of a unique metabolite, dipicolonic acid, which appears to protect them against heat and other agents; this is shed, along with other materials, during this brief germination period.

The second main stage is much longer and has been divided into two subsidiary periods, each of an hour or more. The first is the period of uptake of water, salts and other materials and the second is the phase of true growth, often with elongation of the cell and with a further increase in respiratory rate. By the time it divides the cell has acquired the full enzymic complement of the vegetative condition.

Once proliferation does begin it follows the ideal exponential curve of number/time,  $n = n_0 \cdot e^{kt}$ , where  $n_0$  is the number initially present and  $k$  is a constant. This is therefore known as the logarithmic phase of growth, the logarithm of the number of organisms increasing linearly in time. Virtually every cell is dividing with a steady rate of compound interest, the generation time or doubling time varying from 20 minutes, for many bacteria, to 20 hours for an average protozoon. With techniques permitting a continuous renewal

of medium (Richards, 1934; Novick and Szilard, 1954) the organisms continue to multiply at this constant specific growth rate indefinitely; the renewal-technique also removes a steady percentage of the cells: otherwise they would rapidly tend to the astronomical numbers (p. 11) which must terminate growth under any conditions whatever. Mammalian cells *in vitro* also will proliferate continuously at an exponential rate under suitable conditions (Puck, 1957). Certain enzymes suddenly become active at the onset of proliferation and DNA now increases, in strict proportion to the number of cells (Dean and Hinshelwood, 1959).

Under most laboratory conditions and no doubt also in the wild, the specific growth rate of a colony of microorganisms begins to decline from an early stage, as in the metazoan individual (p. 12). The gross rate of proliferation, therefore, passes through a maximum and the culture enters the phase of growth retardation (Wichtermann, 1953). The curve of size/time passes through an inflexion and the population tends to a limiting size in the so-called stationary phase, of zero net proliferation rate. This limiting population size is statistically constant, and proliferation then exactly balances the death rate among the cells. The limiting number in the stationary state may be maintained for some time, like the size of a culture of tissue cells or of an adult mammal, but usually numbers eventually begin to decrease exponentially, according to the relation  $n = n_0 \cdot e^{-lt}$ ; where  $l$  is a constant and  $n_0$  is the stationary state number; this is the phase of accelerating death rate and is followed by a phase of declining death rate so that the curve is sigmoid, as in the shrinkage of a senescent man (p. 30), and in the degrowth of the thymus. A stationary population size at a new low level is eventually attained. This may then be maintained as long as any food remains—though sometimes with oscillations, and occasionally these are large enough to be regarded as further cycles of growth. They are probably caused in the same way as the oscillatory changes in growth rate common in metazoa (p. 19).

In cultures of *Hartmanella* it was found that the gross proliferation rate was exponential until the organisms began to make contacts with each other and then declined to a linear rate. Agents which separated the cells more widely renewed the exponential rate (Ambrose, 1958). Thus as in the case of metazoan cells *in vitro*, proliferation rate is inversely related to population density and the decline in growth rate is not due simply to the exhaustion of nutrients (though oxygen deficiency may play a part (Lockhart, 1959)), or to the accumulation of toxic excretory products, as once supposed. Also as in the case of tissue cultures, if the medium is filtered off from an ageing culture it will support a new cycle of proliferation by individuals reseeded into it: indeed new cycles may develop spontaneously in an old culture, as already indicated. The decline, therefore, is probably an anticipatory response to deteriorating conditions and leaves ample time, while nutrients remain, for evasive action, for example by encystment (p. 427). The density effect may play a main part in controlling this response and autoinhibitory substances may well be produced. There are

characteristic changes in the cells, largely reversing those during the lag period. There is a decrease in nucleic acid content. Yeast cells in old cultures lose water and therefore become more dense (Richards, 1934), and more resistant to adverse conditions in general. The pattern of respiration changes (Lockhart, l.c.). Permeability decreases, normal enzymic activities "close down," and the cells pass into the resting condition from which they started. Certain special enzymes now become more active. The production of protective dipicolonic acid is one of the final events. Clearly the whole growth cycle is a fully controlled response by the organism.

A particular volume of medium of a specific composition supports a definite maximal population of any one species. Thus the flagellate, *Chilomonas*, proliferates at a rate which is temperature-dependent (p. 415), but the maximal yield of individuals is nearly constant at all temperatures, in a particular medium (Mučibabić, 1956). The maximal number depends on the size of the animal, the medium supporting a fixed biomass. This is illustrated by the observation that *Paramecium aurelia*, which is half the size of *P. caudatum*, produces a maximal population of twice as many individuals, in an identically similar medium.

Two or more successive cycles of growth may occur, not only as a typical oscillatory response but also for quite another reason, owing to the successive utilization of two or more different sources of food as energy (Monod, 1948, 1958). The more amenable food substrate is entirely used up before the second is attacked and there may be a second lag period while the necessary enzymes are being induced (p. 208). This "diauxie" is shown for instance by glucose and galactose as successive substrates for certain bacteria. There are permanent "constitutive" enzymes for the catabolism of glucose, which therefore is the more amenable substrate, while enzymes for galactose must be induced. Enzyme induction (p. 207) occurs in metazoa, though it possibly has not the same importance as this kind of enzyme control in a bacterium.

As already indicated, cell numbers are not always an accurate measure of the biomass of a culture, because cell size varies so much. After the pronounced cell growth of the late lag-period there is a progressive decrease in maximal cell size over succeeding cell generations (Mučibabić, 1956). Later it may increase again (Knaysi, 1951) and finally decline to the low value of the dehydrated, resting individuals. These changes are reflected in the time curves of cell size and of division rate, the peaks of which are not simultaneous. Clearly there is a degree of independence between cell growth and cell division (p. 342) and so cell size may be thrown into oscillation. The partial independence is seen also in metazoa, for instance in some insect larvae, where cell number may be maximal at 20°C, whereas cell size is maximal at higher temperatures (see Bodenstein, 1953).

Some bacterial colonies growing, as they often do, in very crowded conditions on solid media, become as regularly arranged as the cells of a simple metazoan body (Knaysi, 1951, p. 294), with outer, epithelial and inner parenchymatous cells. Occasionally they may grow up as tall columns (see Bonner,

1952). In the so-called smooth colonies of such bacteria as *E. coli* the individual cells are quite separate but in rough colonies they form chains and filaments. A variety of metazoan forms, therefore, readily results from the growth patterns of cells in constrained culture.

It is not a far cry from some of these forms, and from the rather similar palmelloid colonies of chrysomonad flagellates and algae, to the more stereotyped colonial aggregates of the Mycetozoa (Raper, 1941; Bonner, 1952, 1958)

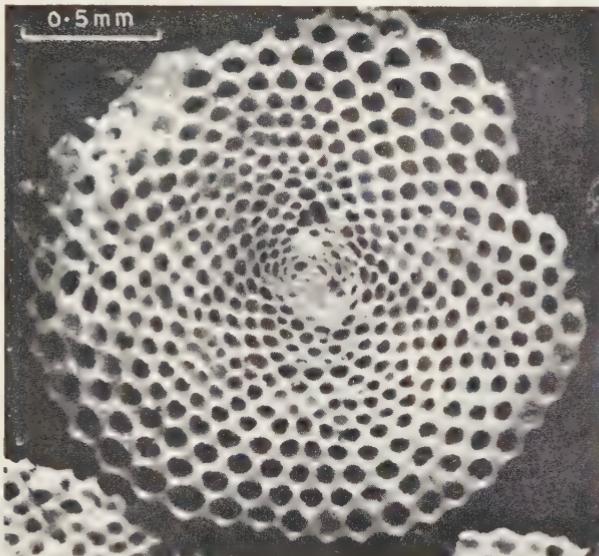


FIG. 9.1. DRIED SKELETON OF THE FORAMINIFERAN, *Nummulites*, TO SHOW THE REGULAR ADDITION, IN SPIRALLY CONCENTRIC SEQUENCE, OF NEW AND PROGRESSIVELY LARGER CHAMBERS

The regularity produces an intersecting system of radially directed spirals. It is established in spite of occasional irregularities, particularly in early life.

(Photo: J. S. Haywood)

which form their specialized asexual fruiting bodies. These have also a high degree of locomotor, and of morphogenetic, integration. The metazoan form is assumed only after growth and proliferation are completed: by suitable conditions of nutrition a reversal may be induced, and proliferation resumed. Here growth and differentiation are perhaps more sharply separated than in any other group of animals.

Permanent protozoan colonies are rarely as highly differentiated as this. The Volvocina form simple plates or spheres, the colonial Radiolaria rather formless masses of identical cells, the Foraminifera simple coenocytia and the colonial ciliates an arborescent habit which permits only very limited further experiments in organization. The growth of some of these is interesting, however. The arborescent ciliates grow monopodially like the gymnoblast

hydroids (p. 44), the apical, oldest zooid having morphogenetic control over the rest of the colony. The Foraminifera, being supported by a calcareous shell, are able to grow indefinitely, with nuclear proliferation, adding new chambers to the shell and maintaining coenocytial continuity through its many perforations. Often the new chambers are added in a remarkably regular order (Thompson, 1942), which varies in the different subgroups. Flat spirals (Fig. 9.1) permit very large sizes: some nummulites reach the size of a florin and species of *Cammerina* a diameter even of 19 cm!

The important lesson to be learned from the study of growth in populations of microorganisms is that there is virtually no situation, certainly none in the wild, in which they grow with complete independence of other cells. Even when direct influence is slight there may be considerable interaction via the medium and its contents. It is not altogether meaningless to compare micro-organisms with blood cells, tissue cells with a fluid matrix. The microbial cell has not only a cycle of growth between cell divisions but a longer cycle by which as a member of a community it ensures a pattern of growth and proliferation which is optimal for the species under the varying hazards of the biological as well as the abiotic environment (p. 407).

The pattern must have long-term controls, and the daughters of each generation continue the trend which was already developing in the previous generation of that population. In their growth potential and in other properties they are significantly different organisms from their immediate parents and offspring. Potentially there is much to be learned about the origin of the metazoan status and the growth of the Metazoa from the study of these long-term cycles of interaction between cells and environments. The growth of the population is controlled with as much precision and teleconomy (Pittendrigh, 1958) as that of a metazoan individual.

## CHAPTER 10

### *The Growth of Individual Cells*

#### 10.1. General

For evident reasons this has been studied mainly on microorganisms. The individual cells of the Metazoa are not easily isolated and in any case they are then abnormal to an unknown degree; *in situ* the growth rate of many is extremely slow and difficult to study. However, some facts have been obtained from isolated tissue cells *in vitro* (Puck, 1957) and quite a lot is known about the growth *in situ* of certain large specialized cells, particularly the oocyte and the neuron. Bacteria may complete a cycle of cell growth in twenty minutes (p. 11), but most Protozoa in 12 to 48 hours, which is also the order of speed of embryonic and cancer cells, and of metazoan cells *in vitro* (p. 89). It is interesting that the pancreas cell produces its own weight of protein in much the same time, 24 hours (Caspersson, 1950), in this case for external secretion and not for growth. Maximal rates are higher in ciliates than in the sarcodines (Richards, 1941). The maximal size reached before the cell divides also seems to be innate, for instance in *Tetrahymena* (Giese, 1957), though in *Styloynchia* and *Blepharisma*, and perhaps in general, it is modified by the type of food and other factors. As already noted, maximal cell size and division rate both vary also with the stage of the population growth cycle.

Growth may be completed long before the cell divides, in some cases as early as the middle of the whole cell cycle, and its size then marks time awaiting division (Wichterman, 1953). In most cases, however, division follows almost immediately, and in some it appears to check growth in full career. These variations may help to explain the different types of growth curve obtained by different workers: (1) a curve with an exponentially declining rate as the main feature, (2) an orthodox sigmoid curve and (3) an exponentially inclining curve with no marked slowing at the end (Kimball *et al.*, 1959), though it is evident that it must return to its initial rate for the next cycle, and that this will make the curve sigmoid. In the yeast cell Mitchison (1957, 1958) found (4) a linear relation between size and time, throughout the cycle, and Zeuthen (1953), using oxygen consumption as the measure of growth, obtained the same relation for protozoan cells. Some bacteria give effectively this same simple relation, probably because these particular species are multinucleate (Knaysi, 1951); they therefore suffer only local, and staggered, checks to their growth owing to nuclear division, and only very infrequent checks owing to cell division. The orthodox sigmoid curve obtained in other cases probably should be regarded

as effectively linear growth, which is necessarily checked each time the cell divides; in those organisms with a markedly linear curve this check must be extremely slight. von Bertalanffy (1949, 1960) has recognized the exponentially inclining curve as being typical of rod-shaped bacteria and the exponentially declining curve as typical of cocci; he attributes this extreme difference to their very different shape and mode of growth, the first maintaining effectively a constant surface/volume ratio as they simply elongate, the second having a ratio which decreases throughout the growth cycle since they grow as a sphere. Kimball (l.c.) also, envisages fundamental differences in the growth mechanism of different microorganisms.

Records for some of the larger Protozoa give less simple graphs, which may be due partly to physiological shape changes during the cycle and partly to differential growth within the cell. This is by no means a simple sac of fluid but is highly differentiated, particularly in the large ciliates. Most of them divide by transverse or by oblique fission and they actively shorten at that time, so that they are relatively short and broad immediately before and after fission. In one case the dimensions change from  $200 \times 56\mu$  to  $176 \times 65\mu$  just before fission, which results in two daughters each of  $88 \times 65\mu$ . In the new cell cycle, length growth starts first, then width growth and finally the third dimension (Wichterman, 1953). In some instances irregularities in cell growth have been attributed to the effect of a rapid growth of the nucleus, during a particular period (p. 133). Starvation of *Paramecium* produces a corkscrew-shaped cell (p. 29) which is an exaggeration of the normal spiral asymmetry, due to differential shrinkage. In *Stentor* the number of longitudinal pigment stripes increases more slowly than cell volume. Cell shape, like maximal cell size, varies during the course of the population growth cycle and under other changes of conditions (Mučibabić, 1956). *Stentor* occasionally grows in length at a time when it should be growing in width (Tartar, 1961).

In the yeast cell even the relationship between volume and weight is differential (Mitchison, 1957). Weight shows the linear curve considered above but volume increases more slowly during the first three-quarters of the cycle and then more rapidly, giving a curve which is quite different in form. Again, bacteria may double their protein content after they have reached maximal size, so that protein synthesis is not rigidly geared to size increase, although in animal and plant cells they generally increase in parallel (R. Brown *et al.*, 1952).

Growing cells typically have a cytoplasm dense with fine ribosomal granules (p. 146), which are basophil in reaction owing to their high content of nucleic acid (p. 220). The nucleus is large and not very deeply staining because the sap/chromatin ratio is high. The nucleolus is variable in size but usually large. The mitochondria (p. 144) are numerous and often large and filamentous, radiating out from the nucleus to the periphery. The appearance is very different from that of typical post-mitotic cells, that is cells destined not to grow and divide again. Some cells, such as those of the imaginal discs of insects, rest for

considerable periods in a primed condition, with large nucleus and nucleolus, but this condition can also quickly develop from the resting state. Within six hours of administering the hormone ecdysone (p. 381) to the bug, *Rhodnius* (Wigglesworth, 1957), the nucleus and nucleolus are visibly enlarging in the relevant cells, and the cytoplasmic basophilia is increasing. Glycogen appears in the cytoplasm (Florkin, 1960; Montagna and Ellis, 1958), and is rapidly used as growth gets under way.

The acinar cell of the pancreas shows these changes at the onset of secretory activity; this is a post-mitotic cell, but it is engaged in productive protein synthesis. There are other cells of this kind and also cells which grow considerably after the last mitosis (Fig. 3.3): two outstanding examples, the neuron and the oocyte, will be considered below. Another striking example is the smooth-muscle cell of the pregnant mammalian uterus which grows from a length of  $50\ \mu$  to one of  $500\ \mu$  or more; this is particularly interesting also in degrowing to normal size again after parturition (p. 28). By contrast the striated muscle cell (p. 84) acquires its giant size by becoming coenocytial and in essence remaining intermitotic. It progressively adds new sarcomeres at the two ends of its fibrils (Picken, 1960). Most of the growth of a blood corpuscle occurs after the last mitosis, that is between myeloblast and promyelocyte stages in the case of the leucocyte (Fig. 8.1).

It was seen (p. 4) that growth of the body continues after differentiation has begun, and in some cases this is true of the individual cytoplasmic unit, for instance the neuron and the striated muscle coenocytium. However, in general it is probably true that continued body growth depends on undifferentiated cells and that these differentiate only when they have finished growth. The erythrocyte does not begin to produce haemoglobin until general growth is complete (Caspersson, 1950), nor do odontoblasts begin to store Ca and P in the cytoplasm for dentine formation (p. 51). Enzymes concerned with the definitive function of the plant cell also appear in the post mitotic stage, when growth is largely achieved (R. Brown *et al.*, 1952). Of course (p. 2) these particular examples are all cases of differential synthesis or accumulation, growth processes rather than pure differentiation.

The Protozoa, reproducing by fission, are cells which must be capable of repeatedly growing after differentiation; there are phases of growth and differentiation in each cell cycle and no doubt some of the irregularities in their growth curves are due to this. In some, differentiation occurs only after the growth of the daughter cells is complete, while in others, it is largely achieved before fission (Tartar, 1961): this is the basis of the classical distinction between architomic and paratomic types, respectively. It is noteworthy that in both cases there is little overlap between growth and differentiation; they are particularly distinct in hypotrichous ciliates (Bonner, 1958), where a new field of ciliature (p. 140) is differentiated in miniature before fission, but grows only after division. Mammalian cells *in vitro* show some tendency towards differentiation in each cell cycle (Willmer, 1958).

## 10.2. The Neuron

Nerve cells are remarkable almost as much for their great post-mitotic growth as for their high degree of specialization as conducting cells. The long cell processes, or axons, of peripheral neurons grow the whole way from the central nervous system (C.N.S.) to their end organs, which may be at the end of a limb. The total mass of the cell may increase  $2 \times 10^5$  times postmitotically (Hydén, 1950, p. 178; 1960). Most of this growth is ultimately in the axon since the cell body or *cyton* grows only  $2 \times 10^3$ -fold and the axon ultimately has between  $10^2$  and  $10^3$  times the mass of the cell body. The rate of linear outgrowth is 1 to 2 mm per day, and as much as 4 mm during regeneration. In the antlers of the stag (p. 18) the rate may reach 12 mm a day (Wislocki and Singer, 1946)!

Growth is assisted by a continuous distal flow of the axoplasm (Young, 1945a) along the centre of the fibre, returning along the periphery and aided by peristaltic movements (Weiss, 1941). This flow continues after the fibre has reached its end organ, since the nerve must keep pace with the further growth of limbs and body. In fact the flow appears to continue indefinitely (Weiss and Hiscoe, 1948; Hydén, 1960), a primitive circulation, maintaining the nutrition and repair of the cell and facilitating new growth in the emergency of regeneration; this is not an uncommon incident in such elongated structures. The rate of excretion of ammonia by the nerve fibre, a measure of its rate of metabolism, also is proportional to the rate of flow of axoplasm, so that the flow is also concerned in the replacement of wear due to the normal functioning of the nerve.

The flow maintains a bulb or cone at the tip of a growing fibre but if the nerve is partially constricted at any point the diameter remains equally narrow throughout the whole of the region distal to that point, while the normal diameter is regained throughout when the constriction is removed (Weiss, 1949). Simple hydrostatic effects cannot explain this phenomenon, which seems to depend on the discrete longitudinal neuro-fibrils, or more probably tubules, often observed in nerve fibres. Presumably constriction closes some of these completely, and leaves others open.

The flow in axons may be compared with cyclosis in plant cells, which likewise serves both growth and general metabolic functions (Steward and Millar, 1954). In more compact animal cells such as *Amoeba*, and fibrocytes, and the spiral organism observed by Picken (1940), the phenomenon leads to overt locomotion of the cell. In crowded cells, and in those Protozoa with a rigid cortex, there can be only cyclosis, as in the typical plant cell. At the multi-cellular level it has its parallel in the peristaltic flow in the stolons of hydroids (p. 414), which, in small isolated pieces, again can lead through growth to overt locomotion (p. 47).

The diameter of a nerve fibre is only  $1/10^4$  of its length (Young, 1945b), and its growth rate therefore is only about  $0.1 \mu$  per day but this is significant. Consequently, the effect of a deficiency of vitamin A, which normally promotes

the enlargement of the nerve foramina of the skull, can be disastrous (p. 299). Fibre diameter does not merely increase in passive correlation with length but also in relation to the number of end organs innervated (J. Z. Young, 1950). Reciprocally, if the nerve diameter is subnormal because of vitamin A deficiency, it is able to innervate fewer end organs. The actual functioning of effector end organs, such as muscles, is found not to be vital for the initial thickening of the nerve (Evans and Vizoso, 1951) but is necessary for the subsequent maintenance of the diameter.

In myelinated nerves the myelin cylinders grow with the nerve and the length of the internodes or sections of myelin sheath is a measure of their growth and of the time since initial myelination. In a regenerated nerve, however, all

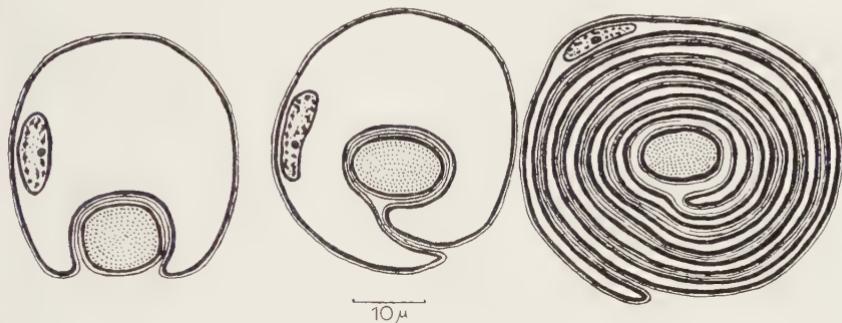


FIG. 10.1. STAGES IN THE GROWTH OF THE MYELIN SHEATH AROUND THE AXIS CYLINDER OF A VERTEBRATE SOMATIC NERVE FIBRE

A fold of the cell membrane of the enveloping Schwann cell extends enormously, to wrap round and round the cylinder. It is not certain if the cylinder itself plays a part in the coiling.

(Based on J. David Robertson (*Waelisch*, 1957))

fibres are myelinated at about the same time and so have all the same internode length (Young, 1945a). The cells of the Schwann sheath, which form the myelin, as a fold or doubling of their cell membrane, follow up the growth of the initially naked sprouts of the axon. The myelin is wrapped round the axon like a Swiss roll (Fig. 10.1) and can thicken progressively by the continued wrapping action of the Schwann cell; this is another fascinating variant of cell growth (Ben Geren, 1956; Schmidt, 1959). It is not yet clear whether the Schwann cell or the axon effects the rotation (J. David Robertson, 1960).

The great power of growth of a neuron is reflected in its size and appearance. Caspersson (1950) and others have been impressed by its resemblance to the oocyte, another cell with outstanding ability for growth. Although post-mitotic, the neuron has a large nucleus and nucleolus, and densely staining cytoplasm, rich in RNA (p. 220). This is used both for growth and for the heavy maintenance of the mature cell. The outstanding growth-promoting ability of extracts of nerve tissue no doubt depends largely on this component,

and similarly the tropic effect of a nerve on the growth or the regeneration of its end field.

Path-finding by growing nerves is a growth phenomenon somewhat comparable in its results to tropistic growth in plants. The method has differences, however; the growth bulb at the tip puts out a number of filiform pseudopodia and the contents of the bulb flow into the one most favoured by orientating forces. These are mainly the stereotactic forces of existing channels and fibres, particularly fibres of the same functional group. Chemical and electrical gradients and possibly other factors may contribute (Weiss, 1941, 1950, 1959). Nerves of anatomical magnitude are formed by stereotactic fasciculation of the individual fibres. End organs attract nerve endings in their vicinity and they also induce the initial outgrowth of fibres from the C.N.S. These actions are relatively non-specific to all fibres in the vicinity; within the appropriate field, sensory or motor, they impose their specificity on any fibres captured in this way (Sperry, 1951). However, it is not possible to interconvert sensory and motor in this way, for the nerve fibres themselves have some degree of prior specification; they tend to innervate particular end organs where possible. *In vitro*, therefore, a heterogeneous group of neuroblasts do not all grow in the same direction but show considerable individuality of behaviour.

It was noted (p. 92) that nerve fibres are more enterprising in their growth than other cells. Nevertheless they do have the same property of thigmotaxis, that is of coming to a halt when they make contact with certain other cells (Weiss, 1941). In this case presumably only a functionally appropriate end cell evokes the response.

### 10.3. Vacuolated Cells

In plants the development of a large intracellular vacuole is one of the major features of growth, but it is unusual in animals. In the stolons of hydroids, when new buds are formed, vacuolation of the cells precedes the onset of mitotic activity (Berrill, 1949a), and persists throughout the phase of proliferation though outstripped by division if this is rapid enough. When division ceases in the mature stolon, the vacuole disappears and the cells become smaller. Tunicate buds also show this feature, and therefore it may occur in the cells of other animals, also. It is interesting that coacervates (p. 148) spontaneously develop vacuoles. The cell vacuole may stimulate growth by mechanically distending the cell, or by increasing its hydration (p. 266). Permanent vacuoles are formed in notochord and other turgor cells. Fat cells reversibly form vacuoles of storage fat, which may grow to an enormous size.

### 10.4. The Oocyte

During its maturation the oocyte or egg-cell grows more than most cells of the body, and in many animals it becomes a giant among cells. That of the newt reaches a diameter of  $1600\ \mu$  (Callan, 1952) while that of some birds, the so-called yolk of the completed egg, reaches a diameter of several inches. This

mass of material is mainly fuel and building materials for the prospective embryo and consists of carbohydrates, lipids, phospholipids, proteins, nucleic acids and conjugates of these, as well as of various quantitatively minor constituents. The cell is, therefore, specialized both in the quantity and in the variety of biological materials it stores. To some extent it serves as a model for all that any cell might be expected to synthesize and as an object of study it offers the technical advantage of large amounts of these materials. Other cells can be studied in the mass, by biochemical methods but the oocyte has much to offer even to direct microscopical, chemical and histochemical study of the individual cell.

It seems that this vast store of material is beyond the biosynthetic capacity of the one cell, and nurse cells of some kind are nearly always found. In most invertebrates these pass large amounts of particulate matter from their own cytoplasm into the oocyte (Wilson, 1928) and in some of the lower Metazoa the nurse cells are engulfed entire (Schultz, 1952). In the higher invertebrates and the vertebrates the nurse cells are small and numerous and form a complete *follicle* round the oocyte. They thus become intermediaries for the transfer of materials from such factories as the liver (Hevesy, 1948) rather than the ultimate donors of material; distribution via the blood stream is essential in this type. The oocyte now receives mainly soluble material but nevertheless much of it is prefabricated and again reduces the burden of synthesis inside the oocyte. Fully functional antibodies and other serum proteins are transferred into the bird's oocyte (Clark, 1960); this process is carefully controlled and foreign antibodies and proteins injected into the hen do not pass in the same way. The follicle cells do more than select what may pass: they have the sole assimilative power and the oocyte is able to acquire very little once it has left the ovary (Clark, l.c.). In some insects primitive nurse cells operate in the early stages and a follicle of small cells later, when most of the yolk or deuterooplasm is being laid down.

The growth of the oocyte of various animals has been described by Wilson (1928), Brachet (1950), Duryee (1950), Romanoff and Romanoff (1949), Callan (1952), Gall (1952), Raven (1948, 1958, 1961) and others. There are three cycles of growth, typically (Raven, 1961), the first being inconspicuous and completed early. In the chick the deposition of a light peripheral layer of fat is the only evidence of synthesis (Clark, l.c.), and the cycle ends before the bird hatches from the parental egg. The second cycle is the main one in duration and biological interest, though not always in the amount of material deposited. Particularly in birds and reptiles the bulk of the yolk is laid down in the third cycle. Moreover it is deposited extremely rapidly, and is packed in from outside, whereas the slower growth of the second period depends more on activity within the oocyte itself.

At the beginning of the second cycle the nucleus suddenly begins to grow and forms the *germinial vesicle*, which controls the subsequent synthesis in the cytoplasm. During the third cycle there is no further growth of the nucleus

(Sturkie, 1954; Witschi, 1956); it is a phase of passive accumulation of deutero-plasm, whereas in the second probably most of the increase is due to "protoplasm", the genetically specific, functional fabric of the cell. The second cycle begins typically, and on the face of it very surprisingly, in the middle of the prophase of the first meiotic, or chromosome-reducing, division of the cell. In the newt it then remains at this stage while the cell grows from  $70\ \mu$  to  $1600\ \mu$  in diameter and the nucleus from  $40\ \mu$  to  $500\ \mu$ . The enlargement of the nucleus is not so great in animals with a more active follicle (Raven, 1961) so that there is a considerable variation in the amount of growth activity by the oocyte itself, even in this cycle. It is noteworthy that even in the newt nuclear growth is considerably less than that of the cytoplasm, but there is probably a second reason for this, namely that the materials produced by the nucleus are mainly of a catalytic nature, effective in relatively small amounts.

The chromosomes reverse their incipient prophase shortening and enlarge, virtually filling the germinal vesicle. This dilutes the DNA which forms the core of the chromosomes, as shown by their feebler basophilia and Feulgen reaction. In the newt the strands of chromosomal DNA become extended as numerous lateral loops (Callan and Lloyd, 1960) which give the whole chromosome the appearance of a lampbrush (Fig. 11.2). These loops are the sites of synthesis of RNA and protein, on which the further growth activity of the cell depends, and probably also of other substances. The material synthesized on the loops passes into the karyoplasm, partly in soluble form, perhaps, but largely as particles which in all known properties are typical nucleoli (p. 137) but are often much more numerous. They or their materials are forced out of the nucleus (Duryee, 1941, 1950) through the pores in its membrane; in the oocyte of the snail, *Limnaea*, the process is assisted by rhythmic contractions and dilations of the nucleus. In other animals the nucleoli pass into lobulations of the nuclear membrane which may constrict off into the cytoplasm; there are also other devices for promoting the transfer (Raven, 1961). The nucleoli grow more rapidly than the nucleus as a whole, which is being continuously depleted by their emigration. Even so the nucleus continues to grow after the chromosomes have reached their maximal size. The secretion of karyolymph, on which this depends, is the function of a special karyonucleolus. In addition to RNA and its associated protein, specific proteins such as alkaline phosphatase, and also glutathione (p. 287), tryptophan and iron, have been identified first in the nucleoli, and later in the cytoplasm (p. 137).

Corresponding loci on the homologous chromosomes behave almost identically in the production of nucleolar and other material and actual proximity of the two seems necessary (cf. p. 388); triploids, in which the trios of homologous chromosomes experience the eternal triangle difficulties, show faulty synthesis activities (Fankhauser, 1954). This requirement for close pairing may be the essential reason why the whole synthesis activity occurs in the early prophase of meiosis, the one occasion when the homologous chromosomes do pair so completely. It seems that the homologous loci must

cooperate in some way in this particular process of biosynthesis. Possibly the pairing does occur at the loci engaged in synthesis in other cells, also, but in most of these few loci are involved at any one time.

Material from the nucleus is first seen, in the cytoplasm of small oocytes, as a pallium of strongly basophil material around the nuclear membrane (Fig.



FIG. 10.2 SECTION OF OVARY OF CRAYFISH, *Potamobius (Astacus)*, SHOWING GROWING OOCYTES, WITH LARGE NUCLEUS (GERMINAL VESICLE), CONTAINING A NUMBER OF NUCLEOLI, AND A PALLIUM OF STAINING MATERIAL PASSED OUT INTO THE CYTOPLASM

On the right is a vacated egg-follicle which has a general resemblance to the vertebrate corpus luteum.

(Photo: P. L. Small)

10.2). This moves progressively out towards the periphery. Later the basophilia and the nucleic acid associated with it become much diluted if not decreased in absolute amount. The RNA becomes masked by other materials which accumulate, and perhaps actually depleted in the process of synthesizing them. There is little doubt that it is concerned with further synthesis by the oocyte, and is the main agent for total synthesis *in situ* but at present it is not certain how soon deposition of imported stores becomes significant and what fraction of the subsequent accumulation is due to this. In most centrolecithal oocytes yolk begins to appear round the periphery of the cytoplasm (Clark, 1960), and the accumulation progresses centripetally. Moreover it is widely held (Raven, 1961) that the newest material lies just under the cell membrane, that deposited earlier being pushed towards the centre. This seems strong evidence that it is fed in from outside the cell; if it had been due to the activity of the pallial material, then deposition would have been expected either to

begin near the nuclear membrane, and to spread out with the pallium, or alternatively to begin at the periphery when the pallium reaches this, and then to spread inwards but with the newest material nearest the centre.

If the interpretation is correct it supports the view that the nuclear material catalyses the synthesis of protoplasm, while the temporarily inert deuteroplasm is fed in from outside. However, the microscopical events are more complicated than this. In telolecithal oocytes the deuteroplasm is deposited mainly towards one side, the inert or vegetative pole, but this is usually the side remote from the blood supply. It is also remote from the nucleus, so that there is no doubt that, whatever their site of origin, some materials move considerable distances inside the oocyte after their initial formation. Static pictures, even a time series of them, are easily misinterpreted and there are still many details of the micromorphogenetic processes to be clarified.

It is fairly certain that the material of nuclear origin forms, or becomes associated with, the small particulate matter of the cytoplasm, the ergastoplasm (p. 146), as in cells generally (p. 333). In addition another cytoplasmic system plays an important part in synthesis there; this is the Balbiani body, sometimes called the yolk nucleus (Raven, 1961). It consists of the components usually known as the Golgi system (p. 149), together with some mitochondria (p. 144), surrounding the centriole (p. 142) and usually lying near the nucleus. The activity of this system may be responsible for a second zone of yolk accumulation which begins near the nuclear membrane and spreads outwards. It is not yet clear how this material differs from that laid down peripherally, but the activity of the Balbiani body seems to be essentially similar to that of the Golgi system of other cells (p. 335), to control the final elaboration of the special products of the cell. In the Porifera and some other primitive Metazoa the whole yolk nucleus of the nurse cells, together with its associated material, passes into the oocyte and possibly continues its activity there, while the oocyte's own system is active early and disappears at the onset of vitellogenesis. This is an indication that it does not "process" the extraneously supplied deuteroplasm.

The great variety of deuteroplasmic materials stored in the oocyte no doubt reduces to a minimum the subsequent biosynthetic work of the embryo. However, for economy and stability in storage most of the materials are aggregated into relatively large granules or droplets which must subsequently be broken down again. In the Amphibia much of the protein, associated with some fat, is laid down as characteristic ovoid yolk platelets, as long as  $35\mu$ . Fat globules vary indefinitely in size. They and some other materials have a stabilizing envelope of protein, which increases the range of size. RNA particles are not usually greater than  $2\mu$  (Clark, 1960) and glycogen granules are even smaller.

The history of the individual constituents is still somewhat uncertain but it is variable and full of interest. RNA is released into the cytoplasm in two phases: the main one, already considered, is the second. The first corresponds to the small, first growth cycle. In view of the progressive dilution or masking

of RNA in the cytoplasm, special methods would be necessary to show if any is synthesized there (cf. p. 332). The dilution naturally is most rapid at the onset of the third cycle, when large amounts of extraneous deuteroplasm are being taken in. A further load of RNA is released into the cytoplasm at the end of the whole growth period when the germinal vesicle breaks down and resumes its interrupted meiotic division.

Although there is no multiplication of chromosomes in the growing oocyte there appears to be a steady synthesis of DNA (Raven, 1961). Even in the early stages there is already twice the amount in a normal diploid cell and in the mature oocyte there is enough for 4,000 to 25,000 diploid cells. This is mainly in the cytoplasm (Hoff-Jørgensen and Zeuthen, 1952), another very unusual feature of this cell. It is probably prepared material for building chromosomes in the young zygote and is adequate to provide for 9 to 10 generations of cleavage nuclei. This is known to be the number of cleavage divisions which occur before the zygote begins to show evidence of synthesizing any DNA of paternal type (p. 133).

Glycogen is one of the earliest deuteroplasmic constituents to be deposited, usually in granular form. It makes up as much as 15 per cent of the egg of *Phascolosoma* (*Golfingia*). The amount appears to decrease later, perhaps owing to the formation of mucopolysaccharides: it is not likely to be used very extensively in respiration which is slow before fertilization. Glycogen is first laid down at the periphery in fishes and in *Phascolosoma* (Raven, 1961), and here some of it may be used in forming the vitelline membrane. In the newt deposition begins round the nucleus, while in *Limnaea* it appears diffusely throughout the cytoplasm. The variation may seem greater than it really is. Vitamin C is synthesized in quantity in the later stages of some oocytes.

In general lipids are deposited later than glycogen (Raven, l.c.), though in some cases peripheral lipid is the first deuteroplasmic constituent recognized (Clark, 1960). Again the initial site of deposition may vary and is described as perinuclear in some, and both peripheral and perinuclear in others. There is reason to think that this material moves about considerably after its initial formation (p. 149). The initial lipid contains a high percentage of phospholipid and subsequently the amount of triglyceride, or neutral fat, increases progressively. From other fields there is reason to suppose that the P-lipid is involved in the mobilization of the triglyceride. Lipid has been found in association with the Balbiani body and other cytoplasmic organelles and a full account of its formation is not yet possible. Acetyl phosphatides, or plasmalogens, an interesting group of derivatives of the phosphatides, are abundant in oocytes, particularly in the region of the Balbiani body.

Protein storage begins later than that of fat, and the whole sequence therefore corresponds to the order in which these three main constituents are used as an energy source by most tissues (Krebs, 1962). It is not the order in which embryos use them, however (p. 261). There is a large number of records showing that deposition begins peripherally and continues there, so that the

earlier material is displaced towards the centre. By this time accretion may be due very largely to the imported elaborated material. Deposition beginning round the nucleus is characteristic of the Arthropoda, but both groups show a secondary zone beginning at the other site. In explanation of this, and of the pattern for glycogen and lipids also, it may be suggested that when the pallial nucleoprotein moves out and spreads, so as to become less conspicuous (p. 125), in fact it comes to occupy a broad spherical shell near the middle of the cytoplasmic shell. All components synthesized then may be deposited on either the outer or the inner surface of the pallial shell, or on both. The relative amounts laid down subcortically and perinuclearly may depend on the final position of the pallial shell, on its previous movements, its progressive exhaustion, and other factors.

A number of enzymes have been detected in oocytes, and the highest concentration of most of them is found before vitellogenesis, or yolk deposition. It has, therefore, been concluded that they are concerned solely with synthesis and are not stored in the protein yolk for the subsequent activities of the embryo: the latter must synthesize its own enzymes. Acid phosphatase is one of the few enzymes found to be still active in formed yolk granules. If there were large amounts stored in the yolk, in the form of inactive precursors, it seems likely that they would have been activated and revealed by some of the experimental treatments which have been used. It also seems that there is a real diminution and not a mere dilution of enzymes. So many enzymes contain the sulphhydryl, SH, group (Barron, 1949) that this may be taken as a general index of enzymic concentration; it is in fact maximal in the early stages and subsequently decreases progressively. The amount of SH-containing proteins tends to change in proportion to that of RNA, a further indication, perhaps, that both are mainly concerned with activity in the oocyte, and not in the subsequent embryo. On the other hand there is an equally striking correlation between their spatial distributions in the developing embryo (Brachet, 1950).

Peroxidases, esterases, alkaline phosphatase, dipeptidases and ribonuclease all are maximal around the onset of vitellogenesis and decrease subsequently. These are a representative group of enzymes, concerned with all the more important groups of metabolite, except carbohydrates, so that the decline may be regarded as quite general. However, these are all catabolic enzymes, and it is becoming increasingly certain (Krebs, 1962) that *in vivo* such enzymes do not promote their reactions in the reverse direction, that of synthesis. The respiratory enzyme, cytochrome oxidase or indophenol oxidase, which certainly is used only catabolically, increases in amount up to a later stage than most, but it also declines eventually (Raven, *l.c.*). This, and perhaps others of the enzymes in question, are concerned in providing the energy for synthesis (p. 257) and this probably accounts for the time course of their activity. It seems possible that the general decline in enzyme activity at the end of synthesis has much the same significance as that in microorganisms which are passing into a resting state (p. 114); if so the enzymes may become inactivated rather than destroyed.

Arginase becomes distributed in graded concentration along the animal vegetative axis (Brachet, 1950), while dipeptidases are more uniformly distributed. Sulphydryl proteins are concentrated round the yolk nucleus and in the cell's nucleus. Acid phosphatase is active not only in the yolk granules but elsewhere in the cytoplasm and also in the nucleus. Dipeptidases are active in the nucleus and increase in amount there to a very late stage, though they do not keep pace with the growth of the nucleus.

Amylase, which breaks down glycogen, and cytochrome oxidase, are located on the mitochondria. In telolecithal oocytes respiration is most rapid in the vegetative hemisphere, in contrast to the condition in the developing embryo, and indicates that it is a function of the deuteroplasm itself and not of the oocyte's protoplasm. There is a sharp increase in respiration rate each time RNA is liberated into the cytoplasm of the oocyte of *Phascolosoma*. Each is correlated also with an increase in biosynthesis. Respiration also changes from the use of the Krebs' cycle, and maximally aerobic reactions, to glycolysis and the gluconic acid shunt (p. 259). The gluconic shunt may have the same significance, for pentose synthesis, as in neoplastic tissues (p. 98).

In the third growth cycle of the avian oocyte yolk is deposited in concentric layers of alternating white and yellow material, probably corresponding to the diurnal cycle of general metabolism (p. 18). The yellow yolk is the richer in glycerides. As already emphasized (p. 123) the material is packed in from the blood stream and the oocyte nucleus now shows no growth. The new adventitious yolk varies with the diet and apparently contains any casual contaminants in the body, including lead (J. Needham, 1942, p. 22), rather as the external secretions do. This yolk therefore seems to be almost as adventitious as the "white", or albumen, and the shell membranes. The ratio of other constituents to water increases greatly, and fat is now the main constituent added; the simple sequence, carbohydrate, fat and protein therefore is not maintained.

The protein content of the blood of a laying hen increases by more than 50 per cent when this cycle begins, but the lipid content increases 250 per cent (Raven, I.c.). Much of the material is synthesized in the liver, which enlarges by 60 per cent at the onset of the laying period in the pigeon. This bird lays only two eggs in a clutch and there is an even more dramatic return of the liver to its resting size as soon as the oocytes are full grown.

There is a growth cycle in the spermatocyte, corresponding to the main, second cycle of the oocyte, but growth is very slight by comparison (Witschi, 1956, p. 19).

The cells of the liver resemble the oocyte in their property of storing material. They similarly store some, for instance fat, round the periphery of the cell, while glycogen and protein are laid down from the perinuclear zone outwards. Glycogen is subsequently mobilized in the reverse direction (Cowdry, 1924).

The main lessons to be learned from the growth of oocytes are first that cells can grow to an enormous size, but probably never unaided. Here there appears to be harmonious cooperation between synthesis and storage *in situ*,

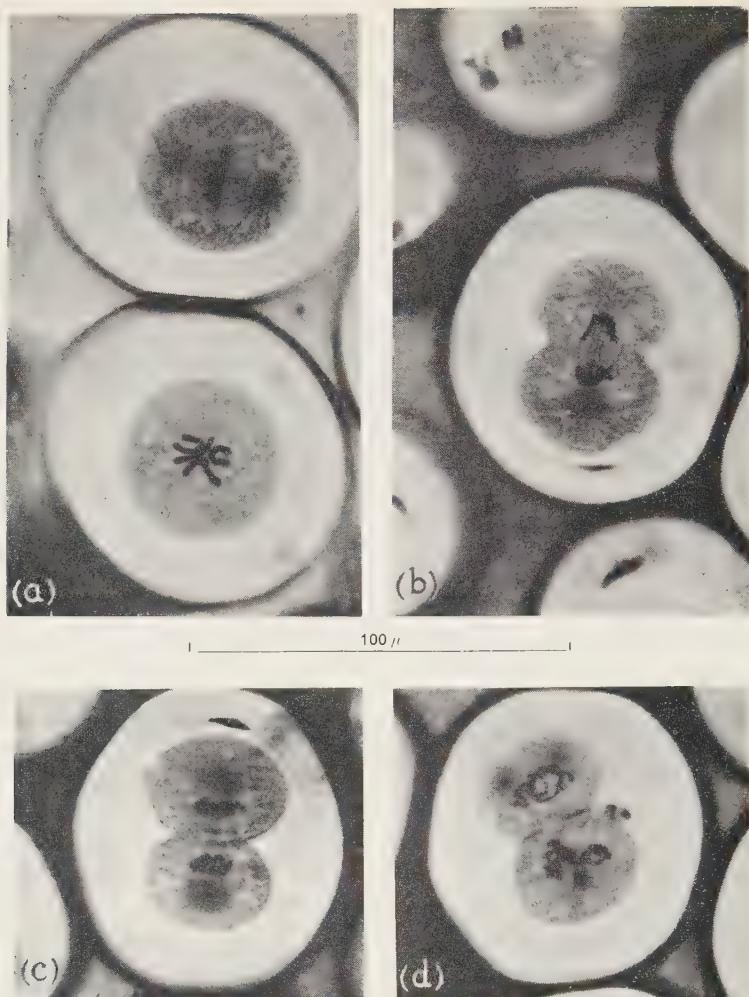


FIG. 10.3. CLEAVING EGGS OF THE NEMATODE WORM, *Ascaris*, TO ILLUSTRATE CELL DIVISION

(a) Metaphase of the first cleavage. The chromosomes are shortened and arranged on the equatorial plate across the centre of the achromatic spindle, formed of the interpolar rays of the two asters. The rays are seen to radiate in all directions from the two centrioles. In the lower egg the metaphase chromosomes are seen in surface view of the equatorial plate. (b) Anaphase. The chromosomes have split longitudinally, and the two daughter groups have moved apart towards the centrioles. The cell itself has begun to divide. (c) Telophase. Cell-division is complete and the daughter groups of chromosomes have formed compact nuclei. These proceed to the next cleavage division at once, without forming a nuclear membrane. (d) Prophase of the second cleavage division. The chromosomes have elongated. The centriole has divided and its daughters are moving apart, to organize the new spindle. It is characteristic of this cleavage in the nematodes that the spindle has a different orientation in the two blastomeres. The egg-shell, vitelline membrane, polar bodies, etc., also are visible.

(Photo: P. L. Small)

and the storage of imported, prefabricated material. The oocyte also shows what a great variety of materials may be assembled in one cell. It demonstrates particularly clearly the activity of the chromosomes in biosynthesis and fairly clearly the roles of the other organelles. It shows how grossly particulate materials and organelles of other cells may be incorporated and perhaps continue to function, a phenomenon still more dramatically illustrated by the spermatozoon at fertilization and by the invasion of viruses (p. 153). The growth of oocytes also is great enough to cause measurable changes in many systemic processes which could not be so easily studied in most other cases of growth.

The oocyte, the neuron, fat cells and certain others show that the normally much smaller and consistent size of cells is not inevitable, although it is around the limiting size for stability in a fluid sphere (Rashevsky, 1960). Presumably cells often differ significantly in their membranes, or in their internal structure, from simple fluid droplets, and the neuron and others differ in shape also. They are probably often metastable, particularly when fed in the manner of oocytes. The presence of many highly organized organelles, as in the ciliate Protozoa, also is likely to affect the limiting size. These organelles show considerable independence in their growth and this must now be considered.

### 10.5. Cell Division

Cell division is essential for sustained growth in all organisms but it is itself a motor activity (Weber, 1958) and not a growth process. It will therefore not be considered in detail here. Its main features are shown in Fig. 10.3, in an ideally simple case, the cleaving egg of the nematode, *Ascaris*. Growth halts at least during the division process itself, and assimilation is checked (Mazia, 1956). In some instances it halts long before the onset of division, which therefore seems to be the trigger causing it to resume subsequently. As a possible factor of this kind, in the control of growth, cell division will be considered in Part II (p. 338). As a physiological process in its own right it has been described and discussed by Hughes (1952, a, b), Schrader (1953), Swann (1952, 1954), Ris (1955), Mazia (1956), and Mitchison and Swann (1958). There is a wealth of information in the recent symposium edited by Gross (1960).

Although in principle cell division is not a growth process it is evident that the cell wall at least must either stretch or grow at this time because of the automatic increase in area/volume ratio when a spherical body divides into two daughter spheres. Mitchison and Swann (1952, 1958) find that there is active growth at the time, most of it in the division furrow. It will be shown (p. 142) that the formation of the division spindle also is a type of growth process.

## CHAPTER II

# *The Growth of Cell Organelles*

ASYNCHRONOUS growth between the parts of the cell is not confined to the Protozoa but is found wherever methods of detecting it are available. Much of it is temporary, since the cell returns to the *status quo* at the corresponding stage of the next cell cycle—or at any rate of the next population cycle. Ehret (1958) distinguishes between the phylogenetic or “synchronous” stability and the epigenetic or “diachronic” plasticity of organelles: this is an interesting qualitative counterpart, at a different order of the time-scale. In cell growth the first question is whether the differential behaviour of the organelles is fortuitous and trivial or whether it plays a significant part in the tactics of growth in the cell. In many cases it is not yet possible to say.

An outstanding example of extreme differential growth within the cell is seen in the Schwann cell which builds the myelin sheath round some of the nerve fibres of Vertebrates. The young cell may be visualized as a somewhat flattened, elongated sack, into which the nerve axon sinks, carrying a double fold of the Schwann cell membrane; this grows indefinitely, rolling spirally round the axon (p. 121). The whole bulk of the myelin is, therefore, due to the great growth of the Schwann cell membrane. The biological rationale for the highly differential rate of growth of this organelle is clear but this is not so in all cases.

The differential growth of organelles indicates some degree of independence between them, but few have yet been grown successfully in isolation. The cell wall of some bacteria can be digested away with the enzyme lysozyme and the resulting *protoplast* continues to grow and divide. However, the rate is only a linear function of time, under conditions which permit an exponential growth rate by intact cells (McQuillen, 1955). Moreover, even so the cell wall of these bacteria is probably a less intimate part of the cell than the cell membrane of most animal cells.

The growth of organelles, therefore, is differential but interdependent. It varies qualitatively also, though there are a number of main features common to the growth of most of them.

### **II.I. The Nucleus**

In intermitotic cells, at least, the growth of the nucleus keeps pace with that of the cytoplasm, and measurements show that a very constant N/C (nucleus/cytoplasm) ratio is normally maintained by them. In postmitotic cells, by contrast, the nucleus rarely grows in proportion to the cytoplasm and often very little at all; this gives weight to the idea that it is nuclear growth which

determines cell division (p. 343). If so, it is the N/C ratio rather than the absolute size which is critical, since enlarged polyploid nuclei are usually associated with a proportionate amount of cytoplasm and this is true of their total masses in multinucleate cells. In those Protozoa with more than one nucleus the gross growth rate of the cytoplasm is proportional to their number. However, the cytoplasm of *Amoeba* has some reciprocal control of mean nuclear size, as shown by implanting foreign nuclei (Lorch and Danielli, 1953). The oocyte is an outstanding example of a cell in which both nucleus and cytoplasm grow to a giant size (p. 122) and the cytoplasm of some insect cells with large nuclei containing polytene chromosomes grows correspondingly large. The maintenance of a fairly normal N/C ratio in these cases is perhaps related to the fact that both parts of the cell are capable of resuming normal division (p. 76); this is not true of all giant insect cells.

The oocyte nucleus grows from  $40\mu$  through  $200$  to  $500\mu$  diameter while the oocyte is growing from  $70\mu$  through  $500\mu$  to  $1,500\mu$ , so that in fact it does not keep pace with the growth of the cytoplasm and the N/C ratio therefore falls. In all eggs, necessarily, there is a period of cleavage of the zygote when the N/C ratio is low but this gradually returns to normal by differential growth at the expense of the cytoplasm. In some cases at least, true growth, the synthesis of new cytoplasmic protein by the embryo, begins just at the time the normal N/C ratio is restored, at the end of cleavage (Fankhauser, 1954).

This involves 8 to 10 cell generations and it is interesting that kappa particles (p. 143) persist for about that number of cell generations after conjugation in those individuals of *Paramecium* which are then lacking the K gene. Again bacteria sensitive to inhibition by alanine will continue to proliferate for eight generations after adding the amino acid. This appears to be the limit of effective action of cytoplasmically transmitted and unrenewed materials, and new synthesis presumably becomes significant at about the same time. Puck (1957) found that under certain conditions even the effect of irradiating cells *in vitro* was not manifest until four or five cell generations later. This may be relevant also to the long latent period in cancer induction (p. 100) and some other delayed responses.

There may be a further small progressive change in N/C ratio throughout the life of the individual metazoon (Minot, 1908, p. 185) just as during the life of a culture of microorganisms (p. 114) and it is clear that the normal N/C balance has the lability which is customary in biological mechanisms (p. 343). For instance nuclear growth can continue if nuclear division is prevented (Peter, 1945; Thomas, 1942).

When the growth cycle of the intermitotic cell is examined more closely the partial independence of nucleus and cytoplasm is seen more clearly. There may be a small decrease in nuclear size immediately after cell division (Richards, 1941) but then it grows rapidly. In other recorded cases, also, most of its growth is achieved early in the cycle, before maximal cytoplasmic growth, for instance in the cells of the larva of the mosquito, *Aedes* (Wigglesworth, 1942), the

amoebocytes of the snail (Wagge, 1954) and the epidermal cells of *Amblystoma* (Overton, 1955). The differential rate is most marked in rapidly proliferating cells (Ris, 1955) and nuclear growth may be late in slowly multiplying ones, again perhaps indicating that cell division is controlled by nuclear growth. The macronucleus of ciliates appears to grow at a linear rate throughout the cell cycle (Cameron and Prescott, 1961). This is, of course, a large, highly polyploid nucleus and, therefore, its growth is perhaps not a one-step process. Apart from the main phase of nuclear growth it seems that there is often a second small spurt just before division (Hughes, 1952a, p. 56; Richards, 1941; Prescott, 1956) which may be simply a preliminary to cell division itself.

In general, labelled materials for a cycle of cell growth go first to the nucleus, as in the growing oocyte (p. 122). In the liver cells of the rat an actual decrease in nuclear size has been recorded after its main growth phase in the cell cycle; this is correlated with a sharp increase in cytoplasmic growth (Laird and Barton, 1954), and this is strong evidence that the nucleus produces essential materials for cytoplasmic growth. This is true also for productive synthesis by the cell: it has long been known that the nucleus, as well as the cytoplasm, shrinks when a gland cell releases its secretion (Minot, 1908, p. 185). In other cells, just as in the oocyte (p. 124), some of the secretion from the nucleus into the cytoplasm may be particulate (Fischer, 1946, p. 48). In the growing oocyte of *Limnaea* (Raven, 1948) nuclear size oscillates repeatedly.

Starvation causes differentially greater atrophy in the cytoplasm (Voegtlín, 1934; Laird and Barton, 1954) though the activity of enzymes may decrease more in the nucleus (Allfrey *et al.*, 1952), perhaps because cytoplasmic enzymes are the more essential for the routine functions of the cell. Sulphydryl compounds are said to favour nuclear growth more than that of the cytoplasm (Voegtlín, *i.c.*; Nickerson, 1948).

Growth of the nucleus within one cell cycle is sometimes polyphasic (Hughes, 1952a, p. 56), each phase involving an approximate doubling of size. This is interpreted as a stepwise progressive increase in polyploidy, chromosome multiplication without nuclear division. Such polyploid nuclei are very common in the Protozoa (Baker, 1948), in insects, in the liver of vertebrates and in the cells of old people (Lancing, 1952, p. 502). As already noted the condition is reversible in some cells of insects. In some of the tissues of the reproductive system, during the proliferations of the oestrous cycle, there may be a regular cycle through polyploidy back to the normal diploidy (Schreiber, 1949). Occasionally there is a 50 per cent increase at a certain step instead of the complete doubling (Salvatore, 1950). During pregnancy in mammals nuclear size becomes a further multiple of that during the oestrous cycle. DNA content is proportional to the degree of polyploidy of nuclei.

In some large postmitotic cells such as those of the salivary glands of the Diptera, nuclear growth may be associated with polyteny rather than with polyploidy; the chromosomes remain diploid in number but become many-stranded and are called giant chromosomes. Either polyploidy or polyteny is

associated with most large nuclei, for instance with those of the prothoracic gland of insects. This endocrine organ secretes the growth and differentiation hormone (p. 381) and therefore it is perhaps not surprising that its nuclei grow even faster than the body in general. The latter may almost double its mass at each moult or ecdysis (p. 21) but the volumes of the prothoracic gland nuclei in successive instars of the bug *Dysdercus* are 12, 45, 152, 468, and  $1,048 \mu^3$  respectively (Wells, 1954). The volume decreases during the later part of each instar, and material is secreted into the cytoplasm, presumably for synthesis there of the hormone. This organ grows also by cell proliferation (Clarke and Langley, 1962).

Giant cells and nuclei are common in insects, nematode worms, Acanthocephala, and other animals. The acanthocephalan body may be largely a syncytium, with a few very large nuclei as much as 2 mm long (Hyman, 1951b, p. 8)! Those of the epidermis may be much branched, their total span being almost equal to the diameter of the body. They may fragment. This recalls the very varied form of the macronucleus of ciliate Protozoa. The nucleus of mammalian polymorph leucocytes also tends to constrict into lobes.

In general the nuclear constituents grow proportionately, and the protein content is closely correlated with nuclear size (Fankhauser, 1954, p. 75; Laird and Barton, 1954). The constituents do not all grow simultaneously, however. The different chromosomes, and even various parts of the same chromosome, replicate at consistently different times (Taylor, 1960). The nucleus of an oocyte continues to grow after the chromosomes have reached maximal size (p. 124), the karyonucleolus continuing to produce karyoplasm or nuclear sap. In view of the large pores in the nuclear membrane (p. 140) (Fig. II.2) it is interesting that the nucleus can enlarge in this way.

### II.I.I. The Chromosomes

The chromosomes are long, helically coiled threads of nucleoprotein, permanently present in the nucleus but shortening prior to division to form a coiled coil, and so becoming denser and more deeply staining. They have deeply staining bands, the chromomeres, due to a high concentration of nucleic acids, mainly DNA (p. 220), and paler inter-band regions with the DN proteins more extended. Classically the protein was thought to provide their skeleton but work by Callan and MacGregor (1958) indicates that continuity depends on the DNA component. Prior to nuclear division the chromosomes become visibly divided longitudinally into two equal chromatids, and some (Gall, 1958) believe that this is the only subdivision short of the ultimate polynucleotide chains, of which there might be as few as one per chromatid. In the region between chromomeres the chromosomes may be as thin as 200 Å, 100 Å per chromatid. Others claim as many as eight subunits or chromonemata, four per chromatid. The number may be variable and in polytenic chromosomes, of course, the number of chromonemata increases indefinitely.

After nuclear division one chromatid of the parent chromosome becomes

the complete chromosome of the daughter cell. It normally duplicates exactly during interphase and two chromatids appear once more. It seems to be literally a duplication (Taylor *et al.*, 1957; La Cour and Pelc, 1958; Hughes, 1958), one daughter cell receiving the parental chromatid and the other a completely new one. Thus, three cell generations after a brief administration of thymidine labelled with tritium, chromosomes are either fully labelled or quite free of label (Hughes, *l.c.*); Taylor *et al.* found after two chromosomal cycles—with nuclear division itself arrested by colchicine, an octaploid nucleus with the label in only one chromatid of half of the chromosomes. All the chromatids were labelled at the first cell division after treatment, but La Cour

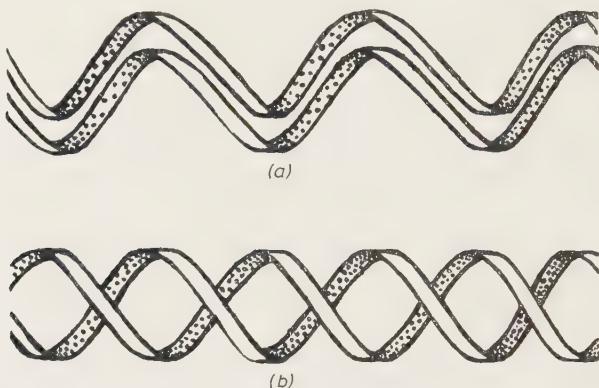


FIG. 11.1. DIAGRAMS TO ILLUSTRATE (a) THE PARANEMIC (ANORTHOISPITAL) AND (b) THE PLECTONEMIC (ORTHOISPITAL) TYPES OF RELATIONAL COILING BETWEEN TWO FIBRES

and Pelc found a number of nuclei at this stage with label in only one chromatid per chromosome so that here there would seem to be two, but no more, replicating strands per chromatid and the daughter chromatid may receive either two new strands or one new and one old. Gall (1958, p. 153) calculated that the total length of a stretched chromosome was as much as 5 cm but that the total length of DNA threads in it was as much as 90 cm. Thus there might be as many as 16 strands—it is likely to be a multiple of two. A diameter of 200 Å would accommodate about this number (Wilkins *et al.*, 1953) and not many more. The different chromosomes may duplicate at different rates (La Cour and Pelc, *l.c.*).

The problem of separating chromatids or other units if they are coiled together orthospirally or plectonemically (Fig. 11.1) is considerable, but theoretically there are ways in which it could be effected without excessive energy expenditure (Kuhn, 1957). The further, coiled-coil condition (provided there were just one gyre per relational twist) could simplify this process (Glass, 1957, p. 764). The concensus of opinion probably favours the anorthospiral or paranemic arrangement for the chromatids because it is a mere topographical

approximation. It has the logical disadvantage of permitting a smaller number of DNA strands to be accommodated in a given space. It is also difficult to visualize how this relationship could be effected during replication (p. 226); at the molecular level, therefore, opinion strongly favours an orthospiral structure, and the chromatids are, in fact, very near this level.

A differential increase in nuclear sap relative to chromosome bulk sometimes occurs (p. 119) while conversely in pycnotic nuclei most of the sap has been expressed (Fawcett, 1958). Heterochromatic regions replicate at a different rate from the euchromatic regions of the chromosome; they are largely concerned with the production of RNA and probably also of protein, for use elsewhere in the nucleus. In addition the ends of the chromosome arms are active longer than the rest (Wimber, 1961), and probably other euchromatic regions also vary in their time of duplication. Chromosomes rather readily fragment, even on simply diluting the medium. This indicates weak secondary rather than covalent bonds (Picken, 1960, p. 130). The broken ends rejoin selectively after mild agents but irradiation tends to break the threads irreversibly.

### 11.1.2. The Nucleolus

One, or occasionally more, permanent or semipermanent nucleoli per diploid set of chromosomes are present in most nuclei, though in large oocytes (p. 124), temporarily, there may be many more bodies of the same type, the secondary nucleoli. Nucleoli are dense aggregates, mainly of ribonucleoprotein, in the form of a reticulum of protein (Sirlin, 1961) packed with granules (Dixon, 1958; Mercer, 1958), which are possibly identical in structure with the cytoplasmic ribosomes (p. 146). The nuclei of the sarcodine Protozoa have no distinct nucleolus but a massive nucleus with its staining material evenly dispersed; by contrast those of the Flagellata have a characteristic vesicular nucleus with little staining matter except in the nucleoli. Sometimes the nucleolus is elongated (Wilson, 1928; Siang Hsu, 1954) and occasionally may be mistaken for a chromosome; other shapes also occur (Raven, 1961) but usually it has a spherical form. Two chemical variants were recognized long ago, a very basophil karyosome and an acidophil plasmosome, the former containing much nucleic acid, the latter more of the proteins with a basic reaction. The primary and secondary nucleoli in the oocyte nucleus of the slug (Cowden, 1958) seem to differ thus in the proportion of the two main constituents. Protein is usually the major component (Vincent, 1955) but lipids, carbohydrates and minerals also have been detected (Sirlin, 1961). In the oocyte the nucleolus contains considerable iron, tryptophan, glutathione and alkaline phosphatase, all of which eventually become abundant in the cytoplasm, also.

The nucleolus typically grows throughout each cycle of nuclear growth and, therefore, is usually conspicuous in rapidly growing and proliferating cells (Caspersson, 1950), for instance cancer cells, and in those which are only growing—the oocyte (p. 122) and neuron (p. 120) in particular. In the newt's oocyte the primary nucleolus grows from  $3.5\ \mu$  to  $15\ \mu$  in diameter and that of *Limnaea*

from 4 to 20  $\mu$  during the main growth cycle. In *Limnaea* its growth is at first more rapid than that of the nucleus as a whole (Raven, 1958). Some neurons have a nucleolus 6,000  $\mu^3$  in volume (Hydén, 1960). The nucleolus also grows when a cell produces new protein for other purposes: there is a new cycle of nucleolar enlargement every time a pancreatic cell resumes its synthesis of zymogen for external secretion. The nucleolus of nerve cells infected with herpes virus enlarges greatly, although the protein produced becomes virus protein. When protein synthesis has ceased, for instance in the postmitotic, myelocyte stage of the leucocyte, and often in the oocyte (Raven, 1961), the nucleolus disappears. In some postmitotic cells (Cowdry, 1952) and in some other cells with a prolonged interphase (Koller, 1947), the nucleolus continues to enlarge, and it is often conspicuous in cells which are resting during certain periods of development.

The nucleolus of the male pronucleus of the rat grows in less than six hours following impregnation of the egg to many times the initial size of the whole sperm nucleus (Vincent, 1955). This rapid growth, which affects the whole pronucleus, is also a dramatic demonstration that the material for nucleolar and nuclear growth can come entirely from the cytoplasm of the moment, since it seems unlikely that uptake from the outside is yet significant.

The single nucleolus of a typical cell develops in contact with a particular heterochromatic region of one chromosome, the nucleolar organizer. In some cells a number of separate loci contribute to its formation (Vincent, 1955) though the nucleonema, a special lateral loop producing the nucleolus, is usually developed only in the one region, the nucleolar organizer proper. Within nuclei of less than a certain critical size there may be a tendency for all such material to coalesce.

In cells producing protein rapidly but not dividing, material passes continuously from the nucleus into the cytoplasm usually in the form of particulate extrusions through the pores in the nuclear membrane (p. 124) and the nucleolus itself is the balance of synthesis over extrusion. In cells which do divide the nucleolus breaks down at that time and the products pass into the cytoplasm (Jacobson and Webb, 1952), the RNA, at least, condensing on the division spindle (Mazia, 1956). This nucleolar fraction therefore may have a special function (p. 328) in the process of cell division itself.

### 11.1.3. The Nuclear Membrane

This is usually reformed in each cell generation and dissolves prior to cell division. In spermatocytes (Barer *et al.*, 1959) it is formed from portions of the ergastoplasm or endoplasmic reticulum (p. 146) which become orientated at first on the whole surface of the individual chromosomes, later retracting or disappearing at the regions of contact between chromosomes. A common membrane then surrounds them all. It lifts off from them as the nuclear sap accumulates. Mitochondria play some part in the process.

Siekevitz (1959a) visualizes the possibility that the nuclear membrane is

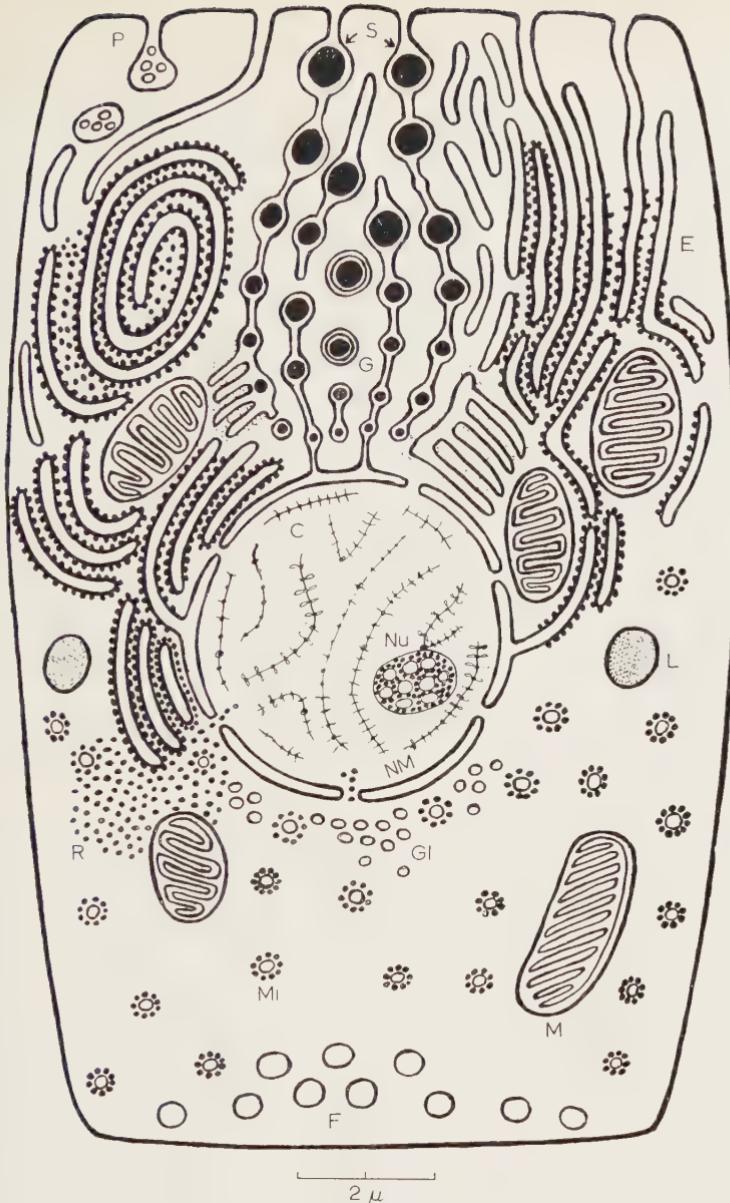


FIG. II.2 DIAGRAM TO ILLUSTRATE THE MORE IMPORTANT COMPONENTS OF ANIMAL CELLS, THE UPPER HALF AS IN A TYPICAL "PRODUCTIVE" CELL, SECRETING EXTERNALLY, THE LOWER HALF AS IN A YOUNG GROWING CELL, AND THE CHROMOSOMES AS IN A GROWING OOCYTE

The organelles are not strictly to scale: C, chromosomes; E, ergastoplasm; endoplasmic reticulum, bearing ribosome granules; F, fat droplets; G, Golgi system; Gl, glycogen granules; L, lysosomes; M, mitochondria, with cristae; Mi, microsomes, small ergastoplasmic bodies with ribosomes; NM, nuclear membrane, with pores; Nu, nucleolus, containing protein and ribonucleoprotein granules; P, pinocytosis (ingestion of fluid drops into the spaces of the endoplasmic reticulum); R, ribosomes, small ribonucleoprotein granules passed out through the pores of the nuclear membrane; S, secreted material. Channels from the extracellular spaces communicate via the endoplasmic spaces with spaces in the nuclear membrane.

(Largely based on Robertson, 1959, and Siekevitz, 1959a)

composed of a mosaic of contributory pieces, homologous with the endoplasmic reticulum (Fig. 11.2); between the pieces are open channels from the nuclear cavity into the cytoplasmic spaces proper. The latter have walls studded with ribosomes (p. 146) and the channels from the nucleus could provide the highway for the nuclear extrusions seen in oocytes (p. 124) and other cells. The nucleus is fairly easily removed intact from some cells and there are no adhering fragments of endoplasmic reticulum; on the present view it would be anticipated that these are cells with few cytoplasmic membranes. Otherwise it would be necessary to assume that all the juxtanuclear pieces of the endoplasmic reticulum are able to cohere and at the same time to break their connexions with the rest of the system. In fact growing cells usually have few cytoplasmic membranes.

## **11.2. Cytoplasmic Structures**

The cytoplasm is rarely less than ten times the volume of the nucleus, except in lymphocytes, and it contains most of the cell's organelles other than the nucleolus and the chromosomes. Most of the genetic information concerning growth, contained in the latter, therefore must be passed out into the cytoplasm. A leading question is whether this takes the form of actual building materials or simply of instructions to cytoplasmic bodies to replicate themselves. It will be seen that in fact most organelles, the subject of this section, do replicate with this grade of autonomy.

### **11.2.1. Flagella, Cilia, and Associated Structures**

Typically flagella and cilia have one or two circles of nine double fibrils surrounding two central ones, all running virtually the full length of the organelle, the outer nine uniting proximally in a cylindrical basal granule or *kinetosome*. This may be connected via a thread or *rhizoplast* with the *centriole*, the initiator of cell division, or with other bodies called *kinetoplasts*, or with both. In the Ciliata the basal granules of each row of cilia are linked by longitudinally or obliquely running threads, the *kinetodesmata*, the whole chain of threads and granules being called a *kinetium*. The whole of this infaciliature forms one of the most highly organized of intracellular structures; it probably includes a conducting, neuroneme system, particularly well developed in the region of the peristome, where the ciliature itself is most complex. The centriole has the form of a cylinder of nine rods or tubules, similar to the kinetosomes.

Both the centriole, at cell division, and the kinetosomes at other times are believed to multiply by fission (Lwoff, 1950). Mazia (1960) suggests that only a certain genetic fraction divides by fission and the daughter germ then promotes the synthesis of the remainder of the daughter organelle. The individual nine tubules may duplicate prior to division of the centriole (Picken, 1960). In ciliates the complete kinetium may be replicated, new kinetodesmata growing out from the one daughter granule of each pair, which remain connected by a transverse thread. In addition individual new cilia or small groups

are inserted into existing rows. Cilia and flagella appear to grow out from their basal granules. The kinetia persist in the Suctoria and in other forms with reduced ciliation, and they develop cilia for the special motile phases of the life cycle.

In most flagellates the flagella replicate only at cell division, but the process resembles the formation of cilia in essentials. The basal granule divides, one product remaining with the old flagellum in one daughter cell and the other producing a new flagellum in the other. Some flagellates, such as *Englena*, have two basal granules, from each of which runs a root to a double-stranded flagellum. The two strands separate at cell division and each reacquires a second granule as the product of an additional division of the centriole; its first division is the usual one, connected with nuclear division. The second centriolar product moves into place at the root of the flagellum and secretes the new second strand of each daughter flagellum (Hyman, 1940). An additional body, the kinetoplast, is sometimes present, and this divides by fission like the kinetosome and centriole. As Grimstone (1961) points out, present evidence does not prove that the basal granules do more than act as organizers for the growth of cilia or indeed of new basal granules either. This would be adequate to explain why strains completely lacking kinetoplasts and other organelles never regenerate them. They may self-copy as the chromosomes do, in which case fission is likely to be an ultimate part of the process, after all.

In the hypotrichous ciliates destruction or removal of part of the specialized ciliation, even of a single cirrus in some species, leads to replacement of the whole (Tartar, 1941), and similar extensive replacements are inevitable during normal fission. In architomous types (p. 119) these occur after fission, but in neotomous species prior to this. The new set may be at first small and the cilia chaotically arranged but they grow and move differentially so as to become regularly arranged in the typical rows. The order is secondarily imposed, therefore, and has not the spontaneity of crystalline regularity.

If a blastomere of a sea urchin egg is enucleated (Lorch, 1952) the centriole continues to divide at intervals for seven hours, producing complete sets of the central apparatus. If the centrosphere itself is removed asters reappear eventually, so that the cell may be capable of regenerating the centriole, unlike most discrete organelles; this question of regeneration of the organelles in general needs further investigation, however, since all visible traces of them disappear reversibly during encystment in protozoa (p. 427). In the budding types of yeast the centriole likewise reproduces by budding (Lindgren, 1952). The centriole appears to reproduce by budding also in sea urchin blastomeres, however (Mazia, 1960), so that the method may be general. The bud then grows to full size, possibly without any further help from its parent. This body promotes the growth also of axostyles and other organelles, in Protozoa, and appears to be a veritable morphogenetic centre, except, perhaps, in the ciliates where the infaciiliature has taken over some of these functions. Mercaptoethanol appears to be a rather specific inhibitor of centriolar division (Mazia, 1960).

The mitotic apparatus (Picken, 1960) or achromatic figure (Mazia, 1956) develops rapidly during cell division, as a pair of astroid condensations usually in the cytoplasm and usually round the daughter centrioles (Fig. 10.3). It grows rapidly, doubling its size in five minutes. By the anaphase stage it has affected a high percentage of the protein throughout the cytoplasm. There is some evidence that a special protein for use in aster formation may be synthesized prior to division (Mazia, 1960) and is not formed if chloramphenicol, a specific inhibitor of protein synthesis, is administered at an appropriate time. The actual formation of the achromatic figure, however, is a process of gelation of a preformed, major protein of the cytoplasm. It involves the formation of hydrogen bonds and of the strong disulphur, —S—S—, bonds (Picken, 1960) and seems to be a typical example of the synthesis of protein macromolecules (p. 162). It is, therefore, a growth process in the sense of producing larger from smaller units but not of increasing the energy content of fabric material (p. 7).

Those astral rays in the axis of the figure become attached each to a daughter chromosome to form the familiar division spindle. By their subsequent contraction the spindle rays help to separate the daughter chromosomes. The astral cycle therefore has important resemblances (Weber, 1958) to the cycle of muscle contraction (p. 172) and is related to growth phenomena in a similar way (p. 5). The contraction phase, which ends in a dissolution of the mitotic apparatus, is promoted by adenosine triphosphate (ATP) and is probably the catabolic phase of the cycle (p. 261). The ready reversibility of the process is an outstanding feature. The contractile vacuole of protozoa and fresh water sponges can probably be regarded as another organelle of this general type, with a regular cycle of contraction and dilation.

The trichocysts and trichites of ciliates appear to have a common ancestry with the cilia; they likewise grow in contact with daughter basal granules and therefore probably are homologous with cilia. They grow inwards from the granule, however, and are stored for use deep in the cortex. The ingenious cnidocysts or thread capsules of the dinoflagellate, *Polykrikos*, definitely appear to arise *de novo* and not by fission, which is precluded by their complexity. However, each develops in contact with a preexisting cnidocyst (Hovasse, 1951) and this may be the model for some other organelles. A thread or desmose connects the cnidocyst with the blepharoplast, or basal granule, so that the cnidocyst also is probably homologous with flagella, in a generic way.

Details of the formation of the nematocyst of the Cnidaria, which is a similar intracellular thread capsule, are still far from clear (Slautterback and Fawcett, 1959). The endoplasmic reticulum probably provides the material and the Golgi complex completes the processing of this, as it does (p. 149) that of simpler products. The complex is attached to the apical pole of the nematocyst throughout its development. In some types of nematocyst the hollow thread develops externally to the cyst and is invaginated later. It is always

preformed and is not simply a condensation at the time of extrusion (Picken, 1957).

### 11.2.2. Self-reproducing Cytoplasmic Bodies

There may be no fundamental distinction between some of these bodies and the organelles of the previous section, but in general they are smaller, more numerous and less concerned with motor activities. They probably reproduce by self-copying, followed by fission. The plastids of plant cells are among the most important because of their function, their highly organized structure and the problem of their mode of growth and proliferation (Mühlthaler, 1955; Grimstone, 1961); they may undergo fission. They are of interest as the classical example of bodies inherited purely cytoplasmically (Darlington and Mather, 1949, p. 171). Pyrenoids in *Eudorina* and the photo-receptive stigma of *Chlamydomonas* also are believed to multiply by fission (Hall, 1953).

In animals the best known example of a self-producing cytoplasmic particle is the kappa particle of killer strains of *Paramecium* (Sonneborn, 1948) which produce a substance paramecin, lethal to individuals of sensitive strains. The kappa particle is only self-multiplying in the presence of a dominant killer gene, *K*. A certain number of individuals, homozygous for the recessive, *k*, but inheriting kappa particles cytoplasmically from one parent, arise at conjugation, but these produce no more kappa particles and those initially present are diluted out in 7 to 8 generations (p. 133). In the presence of *K*, however, kappa can build up to normal strength again even after dilution to the limit of one particle per cell. The normal strength is 200 per heterozygous cell, *Kk*, and 400 in *KK*, a particularly interesting example of quantitative precision in the genetic control of growth (p. 385). Kappa multiplies throughout each interphase, but most rapidly during the first cell cycles after conjugation, the number returning to normal again after 7 to 9 generations (Chao, 1953), when the new genotype becomes established. This has its parallel in the cleavage of the metazoan egg, and elsewhere (p. 133).

The multiplication of kappa is not rigidly geared to that of other cell constituents; this is one of the many instances of such flexibility (p. 343). It is possible to speed up general growth and division, even in *K*-bearing individuals, to such an extent that some progeny come to lack even a single kappa particle. These are thenceforward non-killers, or more correctly they are innocuous, notwithstanding their *K* genes, for these cannot produce kappa *de novo*. For this and other reasons it is suspected that kappa may be a virus-like, foreign body. However, there are examples of genuine indigenous particles, such as chloroplasts, being permanently lost by dilution out, and arising only from homologous bodies of the cytoplasm. Dilution through differential synthesis is recorded also for the melanin granules of iris cells (p. 89), liver glycogen granules, the *colloid* of the thyroid cells, and the chondrin of cartilage cells. This is differential growth at the molecular level (p. 2).

Pigment granules are usually discrete cell inclusions, of the size range under consideration. The chromoplasts of the insects *Bombyx* and *Drosophila* contain all the enzymes necessary for pigment formation and are also self-reproducing (Kikkawa, 1953). By contrast, vertebrate melanin granules, the most studied among the pigment granules of animals, are thought to be synthesized *de novo*, by the deposition of melanin from solution or suspension (Raven, 1953), on to the matrix of a protein vacuole which may originate (Montagna, 1958) in the Golgi system (p. 149). Without this matrix it remains in suspension (Plummer and Kopac, 1953). The unpigmented matrix probably forms the colourless granules found in albino hair (Barnicot and Birbeck, 1958). The final granule is about twice the diameter of the initial matrix vacuole and varies from  $0.05\ \mu$  to  $0.2\ \mu$  in different animals, but is uniform in size in any one species (Dalton and Felix, 1953; Becker, 1953). The matrix probably does not originate from mitochondria as once supposed, since the latter have such a characteristic structure (see below) quite unlike that of melanin granules.

In mammals the mature granules are passed from the dendritic formative cells into the epidermal cells of the skin and hairs (Fig. 4.23), where the pigment can show to best effect; this is an interesting example of cellular interactions in growth (p. 91). It is probable that the machinery for synthesis itself can pass from melanogenic cells to their albino counterparts at the edges of white patches in piebald guinea pigs (Medawar, 1953). The dendritic cells so infected then proliferate true to their new melanogenic condition.

Cytochrome-containing granules are self-reproducing and if completely lost from the cell are not regenerated *de novo* (Tobias, 1959). Like kappa particles they are also under genetic control for proliferation, and mutants unable to synthesize the pigment have been obtained by irradiation. These cytochrome particles probably do belong to the mitochondrial system.

### 11.2.3. Mitochondria

In contrast to most of the organelles of the previous section mitochondria are probably universally distributed, at least in aerobic cells. They are indispensable organelles and have a highly organized and characteristic structure, as revealed by the electron microscope. They are spherical or filamentous,  $0.5$  to  $3.0\ \mu$  in diameter, consisting of a double-walled limiting membrane, the inner lamella of which is infolded at intervals (Fig. 11.2) forming transverse or oblique plates, the *cristae* (Bradfield, 1953). Their function is to carry the respiratory, *cyclophorase*, enzyme system of the cell (Green, 1951) and some other enzymes. They are very mobile in some cells, but not in muscle and kidney cells, where their function may be sharply localized. They are largely composed of protein and lipoprotein and have characteristic staining properties.

With so many clear labels it should be relatively easy to trace the growth history of mitochondria but their size range overlaps that of other bodies, some of which have not yet been characterized, and their diagnostic internal structure is not evident at the low magnification necessary for much of the work.

Biochemical work, depending mainly on differential centrifugation, separates only by size and weight. The general opinion is that they are self-reproducing bodies which never arise *de novo* from molecular-sized precursors (Hackett, 1955; Brachet, 1957) though Hagenau (1958) suggests that certain smaller, dense bodies may be mitochondria developing in this way and Oberling (1959) described the development of *cristae* in them. Mitochondria continue to proliferate as discrete bodies when isolated on to the chorioallantoic membrane of the chick (Le Clerc, 1954). They grow mainly in length, like typical bacilli (p. 118), and divide by transverse fission. There is probably multiple fission in many cases, since small spheres and long filaments are more common than intermediate forms.

Some workers have concluded that the filamentous form is most characteristic of rapidly growing cells (Dalton and Felix, 1953; Shafiq, 1954; Willmer, 1958) and others the small granular form (Woods, 1949; Chantrenne, 1952*b*). Probably both are common, the predominant type depending on the relative rates of growth and fission. Dalton and Felix (1953) found only the small granules at the time of cell division, and both forms during interphase, so that there may be a regular cycle during each cell generation. Liver cells often have filaments centrally and granules at the periphery (Cowdry, 1924), while the intestinal cells have rods distally and granules basally (Al-Hussaini, 1949). Probably both are healthy, functioning bodies, since unhealthy mitochondria become vesicular (Porter, 1954) and clump together (Bourne, 1951, p. 233; Cowdry, 1952, p. 381).

There is no doubt that new mitochondria are rapidly recruited in growing cells of all kinds (Ludford, 1951; Bourne, 1951), and in response to increased food intake (Cowdry, 1924) or to the administration of anterior pituitary growth hormone, APGH (Greenbaum *et al.*, 1954). They proliferate also when a virus invades the cell. The proliferation of mitochondria tends to run ahead of the growth of the cell as a whole and, by analogy with other agents, this probably implies that they are important in promoting growth in general (p. 330). There is the same implication in the fact that their numbers dwindle in mature granulocytes, though these are short-lived cells and conceivably the paucity of mitochondria might be related to the brevity of their functional period rather than to the cessation of growth.

Although reproduction by growth and fission seems well established as the usual method of mitochondria there are further indications that *de novo* synthesis also occurs (Oberling, 1959). In a growing culture of the flagellate, *Polytomella*, Jeener (1952) recognized small particles, in the size range of the microsomes (p. 146) which appeared to grow progressively into the mitochondrial range (Chantrenne, 1952*b*). Others (Siekevitz and Zamecnik, 1951; Szafarz, 1952) have recorded maximal uptake of labelled materials into the smallest granules of the cell and later found most of the label in the progressively larger particles, up to mitochondrial size. This could mean simply that all particles are taking up the raw materials, for whatever purposes, and that there

is a slower uptake in proportion to size, for some quite trivial reason; there is also the further possibility that raw material for general synthesis in the cell is passed along a production line constituted by these particles (p. 333) and does not measure their own growth (p. 147).

In incipiently cancerous liver cells, which lose the normal catalase activity of liver cells (p. 97), the decrease in activity begins in the microsomes and only later is evident in the larger particles (Greenstein and Meister, 1952). When the particles from a mass of cells are separated by differential centrifugation a number of properties, such as the relative proportions of proteins, nucleic acids, lipids and other constituents show a fairly simple gradation with particle size (Dounce, 1950, 1951; Keller, 1951; Pollister, 1954) which also would be consistent with the size gradient being a developmental series as well. However, much of the smaller particulate matter has its own discrete identity, and a structure distinct from that of the mitochondria.

A number of chemically distinct types of particle within the total size range of the mitochondria have been separated (Munro, 1954; Laird *et al.*, 1953). The most discrete and important are the lysosomes of de Duve *et al.* (1955), having the size of small mitochondria but not the crista structure, and bearing a functionally characteristic set of enzymes, lytic in nature: ribo- and deoxyribonucleases, cathepsins,  $\beta$ -glucuronidase and acid phosphatase. They are sharply distinct from mitochondria, therefore, in everything but size; unfortunately little is known of their growth and proliferation. They are almost certainly important as agents in degrowth processes, and possibly also in positive growth.

Robertson (1959) and Grimstone (1961) have considered the possibility that mitochondria are formed in quite a different way, as pinched-off invaginations of the cell membrane. This illustrates the degree of uncertainty which exists, and must remain, with only static pictures as evidence.

#### **11.2.4. The Ergastoplasm or Microsome Complex**

Claude (1940, 1954) isolated from liver cells by differential centrifugation, after the larger particles had been removed, bodies of the size range 50 to 300  $\mu$ , with distinctive properties. This fraction included all the significant finer structural components of the cell, the remaining supernatant containing only the ground cytoplasm together with some free ribosomes (*see below*), and materials in simple solution. Electron microscopy has shown that these microsomes are fragments from the breakdown of a continuous endoplasmic reticulum found in liver and other cells. This consists of paired lipoprotein membranes, with their apposed faces "clean" or "smooth" and the opposite surfaces studded with small 10 to 15  $\mu$  granules of ribonucleoprotein (RNP). For these Roberts (1958) suggested the term *ribosome*. These are very similar in all cells (J. Bonner, 1961). Their RNA is mainly on the surface or at least is accessible (Petermann and Hamilton, 1961), in contrast to the structure of viruses (p. 153). For the whole labyrinthine system of membranes with

ribosomes Hagenau (1958) suggests the historically sanctioned term *ergastoplasm* (Fig. 11.2). The spaces between the membranes of a pair enlarge in places to large cisternae, and they also probably communicate directly with the spaces of the Golgi system. The membranes of the latter are smooth on both surfaces; they contain more lipid and differ in other respects from the general reticulum. The membrane system is best developed in productive cells such as the pancreas acinar cell (Mercer, 1959) and is virtually absent from rapidly proliferating cells (Slatterback and Fawcett, 1959); these have instead finely granular material, much earlier called microsomes by Wilson (1928, p. 356) and probably consisting of ribosomes on a small nucleus of lipoprotein, or lying quite free. Free ribosomes occur also in the glandular type of cell, between the pairs of membranes. It seems possible that on centrifugation the ergastoplasm breaks up along natural cleavage planes, so that Claude's microsomes do not differ fundamentally from those of Wilson. In secreting cells they may be specially organized so as to facilitate segregation of secretory products within the clean labyrinth, which also communicates with the exterior (Fig. 11.2). The granular labyrinth represents the true interior of the cell and is in open communication with the nuclear cavity. The membranes differ from those of the mitochondria in lacking phospholipid (Siekevitz, 1959a).

The mode of growth of the ergastoplasm is somewhat uncertain. Porter (1954) found particles of microsomal size in process either of fission or of self copying. Fission would seem impossible in the membranous state, but the possibility of natural fragmentation is important here. The cloud of material often seen round the nuclear membrane (Fig. 10.2) is probably new ergastoplasm, either secreted entire from the nucleus or elaborated *in situ* on a foundation of material so secreted. It has been suggested that the general cytomembranes are formed by a simple continuation of the process by which the nuclear membrane itself was produced (p. 138). RNP granules of ribosome size are found associated not only with the nucleolus (p. 137) but also with the chromosomes (Hagenau, 1958). Some workers believe that either the Golgi system, specialized as a *nebenkern* in some cells, or the mitochondria, or both, are concerned in the synthesis of ergastoplasm, and this might explain the presence of mitochondria round the newly forming nuclear membrane (p. 138), though there are possibly respiratory reasons for this (p. 262). Others believe that the ergastoplasm can form anywhere in the cytoplasm, though probably always in relation to precursors from the nucleus. At the onset of a new phase of growth in yeast a number of small bodies appear (Ashikawa, 1958) which are probably intermediate stages in the formation of mature ribosomes; almost certainly these are initially formed independently of the lipoprotein membranes.

The use of labelled raw materials (p. 145) indicates that uptake is at first most rapid in the supernatant cytoplasm and later in the particles (Sacks, 1953, p. 217; Greenstein and Meister, 1952), but for the reasons previously given it seems unlikely that the latter simply condense from ground cytoplasm. The picture is again complicated by the fact that the ergastoplasm is, in turn, an

agent for general synthesis by the cell. The incorporation of amino acids into the system is concerned mainly with this (p. 192), and the simultaneous co-operation of the mitochondria is necessary. Very brief labelling is essential for accurate tracing (Roberts, 1958). Synthesis by the system is rapid (Brachet, 1957), the products later being liberated into the supernatant, but the synthesis of the system itself is much slower (Roberts, l.c.). There is a considerable time lag before labelled materials appear in the permanent fabric of the ergastoplasm. This is to be expected if the ribosome materials are synthesized in the nucleus and must then make their way out into the cytoplasm (Roberts, 1960).

After enucleation of *Amoeba* there is a rapid decline in activity of enzymes known to be constituents of the ribosomes (Cohen, 1959) so that the nucleus seems to be a permanent necessity, for the maintenance of these particles as well as for their initial growth. At the same time they do appear to grow in the cytoplasm, from units which are perhaps synthesized only in the nucleus. By centrifugal fractionation of the ergastoplasm at different stages of a bout of synthesis (Roberts, 1960; Britten, 1962) it has been shown that these ergastoplasmic bodies grow progressively through "eosomes" and "neosomes" to the mature ribosomes. These are known respectively as the 14S, 30S, and 50S particles, in terms of their centrifugal properties, the 14S particle probably being the form which leaves the nucleus. A 30S particle has a weight around 700,000 Daltons and consists of three RNA molecules each of M.W. 120,000 and 30 molecules of a basic protein, each 1/10th of this size (Petermann and Hamilton, 1961). The weights of the larger particles are not exact multiples of those of the smaller, so that fractions are lost or other constituents are gained during polymerization. The larger particles also fragment and start new cycles. After a period of starvation the multiplication of ribosomes is greatly accelerated and approximates to an exponential rate (McQuillen, 1961). This seems to indicate that the cycle of multiplication in the cytoplasm sets the pace, since synthesis in the nucleus is likely to proceed at a linear rate.

The ribosomes are naturally very unstable to ribonuclease (RNAase), and they are unstable also to starvation, which causes them to clump. The Mg<sup>++</sup> ion and a dialysable factor, possibly ATP (p. 318), normally stabilize them. However, some preparations of ribosomes *in vitro* have been found to develop spontaneously into large, 1 to 5  $\mu$ , artefact vesicles, or "protomorphs," which are very stable under many conditions (Roberts, 1960). They are more stable than de Jong's coacervates (de Jong, 1949; Booij and de Jong, 1956; Oparin, 1957) which they otherwise resemble, in their spontaneous formation and in other properties. They are capable of incorporating amino acids and show other vital properties. They are important in emphasizing the wide morphogenetic potentialities of the ergastoplasm system.

Coacervates range from 2 to 670  $\mu$  in size, considerably overlapping the range of size of cells, whereas protomorphs are of the mitochondrial size range. There is reason, therefore, to think that discrete bodies of the whole

cytological range can form spontaneously. Coacervates incorporate further materials selectively, even from very dilute solution, and they grow and show other properties of living microorganisms. Like the protomorphs, therefore, they are very relevant to the study of growth at this level. At high temperatures Fox *et al.* (1959) have produced similar spherules, in the 0·4 to 1·4  $\mu$  size range, from synthetic peptides.

The importance of these bodies is that they spontaneously appear from a colloidal solution, as one or more separate phases which are not in static equilibrium and that the phase boundary has selective permeability. These are necessary basic properties, and possibly even sufficient properties, for a primitive living system and the significance of the bodies, therefore, goes beyond growth alone. Under certain conditions they spontaneously develop vacuoles and other separate phases inside, and they show streaming processes reminiscent of those seen in many living cells. Living systems must produce membranes and other structural components in the same general way as these coacervate bodies (Picken, 1960, p. 190).

### 11.2.5. The Golgi Complex

As already suggested (p. 147) this may be essentially a specialized region of the ergastoplasmic system, concerned with the elaboration of special products for use outside or inside the cell. It is conveniently considered here, therefore, although it is often one of the larger organelles. Like the general membrane system (p. 147) it is poorly developed in young rapidly proliferating cells (Willmer, 1953, p. 12) but is best developed in such productive cells as the oocyte and spermatocyte, the cells of the mammary gland, pituitary and adrenal cortex, melanoblasts, enamel cells (ganoblasts), chondroblasts, keratinizing cells and neurons. In the spermatocyte it helps to form the acrosome, in oocytes probably a variety of materials for the future embryo, in the neuron the materials essential for the heavy work function of the axon, and in other cells their characteristic product. Free fragments of Golgi vesicles have been recognized in milk. It is therefore an agent in the productive type of growth and its own growth may have meaning only in this capacity. It is involved in the elaboration of vitamin C, fats, phosphatides (Bourne, 1951) and mucin (Florey, 1955) and no doubt other materials.

Consistently with this picture, the duplication of the units produced by the system has never been demonstrated; more are always recruited *de novo*, from small precursors. This is in contrast to the other organelles, and is another reason for considering the system last. The specific product of any particular cell is first segregated as suggested in the smooth labyrinth of the ergastoplasm and passed to the Golgi zone for final elaboration as discrete bodies, with insulating lipid coats. The bodies grow considerably in the Golgi zone (Fig. 11.2). They may be stored for a time in the cell, sometimes filling the cytoplasm completely (Fig. 11.3). The Golgi complex waxes and wanes in parallel with the productive functions of the cell. In the adrenal cortex the relevant

hormone, ACTH (p. 370) causes its enlargement, and in the neuritic disease, beriberi (p. 309) it becomes enlarged in neurons.

The complex is often associated topographically, and probably functionally, with the centriole, particularly in the oocyte, and with the parabasal body in

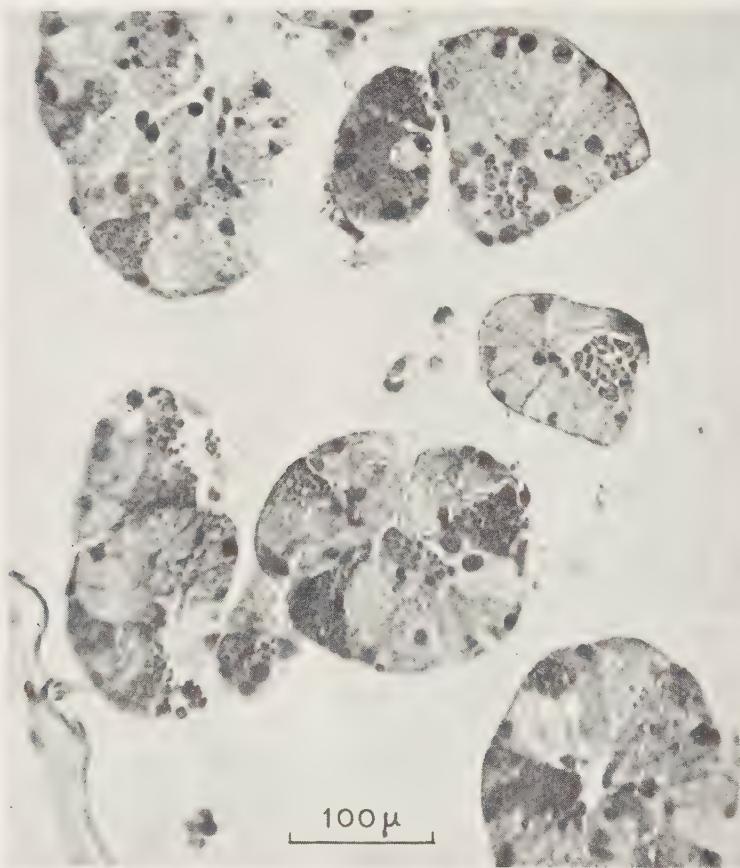


FIG. II.3. TRANSVERSE SECTION OF A GROUP OF ACINI OF THE SALIVARY GLAND OF THE COCKROACH TO SHOW SOME CELLS PACKED WITH ZYMOGEN GRANULES AND OTHERS EXHAUSTED

Discharge appears to be all or none in any particular cell.

(Photo: J. S. Haywood)

flagellates (Grimstone, 1961). The centriole, therefore, may play some part in the control of secretory and related activities, as well as in the production of internal structures such as axostyles, cilia and flagella through their basal granules. In addition, it controls cell division and conceivably also, through the rhizoplast, the movement of cilia and flagella. Secretion, as a biochemical effector function, seems to be the natural link between the physiological effector

functions and synthesis more generally. The same range of activities is probably shown by the Golgi system, since it has a sharp increase in activity just before cell division. The wide range also strengthens the view (p. 5) that the work functions have much in common with the growth processes. The Golgi system is particularly associated with the formation of fibrous proteins (Harris, 1948), which play a large part in motor activities and other functions. Alkaline phosphatase is regularly associated with the formation of fibrous protein (Danielli, 1951, pp. 189, 195) and is often highly concentrated round the Golgi zone.

### 11.3. The Cell Membrane

There is now very good knowledge of the growth of the plant cell-wall (Preston, 1952; Frey-Wyssling, 1953; Picken, 1960) but this is an extra-cellular product, comparable to the cuticles and other exoskeletal structures of the Protozoa (Hyman, 1940; Grassé, 1953). As yet little attention has been given to the true cell membrane, plasma membrane, or plasmalemma of animal cells (Roberts, 1961). Until the advent of the electron microscope it was scarcely possible to identify a specific anatomical and physiological membrane, much less to measure its growth, though it has always been evident that one must exist and that it must grow approximately 30 per cent in every cell generation, so long as the average cell size remains constant.

It has been a familiar fact for some time that if the membrane of a typical cell is ruptured in a suitable saline medium it can be rapidly repaired by a "surface precipitation reaction" of the cytoplasm which begins to flow out (Heilbrunn, 1952). This seemed to imply that the membrane was a labile transient structure and, therefore, not subject to growth in the usual sense. Similarly some have supposed that the plasmalemma of *Amoeba* might be continuously resorbed posteriorly and redeposited anteriorly, when the animal moves, as the cortex below is known to do; this is a purely physiological cyclic change, and one of differentiation rather than of growth. Studies of membrane permeability indicated a highly organized structure, a double lipid layer between two protein layers (Davson and Danielli, 1952), yet laboratory preparations of model paucimolecular films of similar materials indicated that such structures would form spontaneously at the cell surface and at any other interface.

The present picture of the cell membrane is not very different from this: indeed electron micrographs have confirmed the essential structural features predicted from studies on permeation, but it now seems probable that it is a more stable and permanent structure than previously supposed, and that it enlarges by a true growth process. It is continuous with the endoplasmic reticulum (Fig. 11.2) and is likely to have a similar mode of synthesis. Positive evidence of this has been revealed by Porter and Machado in plants and by Mercer and Wolpert in animals (Roberts, 1961). They describe tubular structures, derived from the cisternae of the endoplasmic reticulum, fusing to

form the extension of the plasma membrane which occurs in dividing cells. There seems no good reason why the cell membrane should not also be extended by the endoplasmic reticulum growing and "paying out" on to the surface subsequently. The surface precipitation reaction may prove to be such a herniation of the endoplasmic system. The mode of growth of the plasmalemma in cells lacking any endoplasmic reticulum calls for further study.

It is thought that all true growth occurs during interphase, the increase in surface which manifestly occurs during cell division being entirely by stretching (Roberts, *l.c.*). The tangentially disposed protein component may be expected to possess considerable extensibility, while the radially arranged, discrete lipid molecules may remain as a definite, stable double layer through being bonded to the overlying protein molecules. The resulting tension in the membrane will be resolved by the subsequent interphase growth.

It has already been emphasized (*p. 121*) that the myelin sheath of vertebrate somatic nerves is essentially a fold, that is a double thickness, of the cell membrane of the Schwann cell, enormously extended by growth and coiled round the mature axis cylinder (*Fig. 10.1*). This is the clearest instance yet known of the capacity of the membrane for sustained systematic growth.

#### 11.4. Other Cell Structures

There remain a number of cell structures which for a variety of reasons will be considered separately (*Chapter 13*). They are more restricted to particular cells than most of those already considered and also a number are deposited extracellularly. They may have a characteristic texture but usually not a discrete form, often varying in size from the molecular, indefinitely upwards. Most important for the present purpose is that their mode of formation is different: it is a process at the molecular rather than at the morphological level. These materials bridge the gap between the growth of the smallest morphological entities, the organelles, and the synthesis of the smallest protein molecules.

The gap is one in the pathways of synthesis rather than primarily a gap in the size hierarchy, for there are molecules such as that of haemocyanin, with a diameter as great as 200 Å, overlapping the size range both of the universal cell organelles and of these specific cell products. Again, substantial amounts of the supernatant cytoplasm can reversibly change *en masse* from a sol to a gel condition; there is reason to think (*pp. 5, 47, 120*) that this change is, in fact, relevant to the growth of the kind to be considered in *Chapter 13*. Like the latter it is a change in what is called the tertiary grade of molecular structure (*Cohen, 1959*).

## CHAPTER 12

*The Growth of Viruses*

VIRUSES are intracellular parasites, their infective body usually having a very characteristic shape (Fig. 12.1), with a maximal dimension varying between 10 and 250  $\mu\text{m}$ . Collectively, therefore, they cover the range of size between ribosomes and melanin granules. In many cases they break down and reproduce as smaller units, which eventually recombine to form the mature infective bodies. The initial phase is virtually growth at the molecular level, therefore. Their mode of reproduction is probably in some respects peculiar to them and for this reason it is better considered separately from that of the indigenous organelles. Another reason for separate treatment is that much more is known about most aspects of virus reproduction. This is partly because their economic and clinical importance has attracted a large amount of very penetrating study. It is also because as foreign bodies and parasites they carry unique natural "marker" properties, their infectivity, their serological and genetic specificity, and chemical markers, such as unusual bases in their nucleotides. The best example of this is hydroxymethyldeoxycytidine in the bacteriophage T<sub>2</sub>.

In many ways viruses are ideal objects for study. For instance their reproduction rate is often fantastically rapid, by general standards. The individual generation time may be as short as 35 seconds (Latarjet, 1952) and the bacteriophage type of virus sometimes multiplies a hundred-fold or so, say seven generations, in an infection cycle lasting only 13 minutes of which, even so, the first few minutes constitute the usual preparatory or lag phase and the last four are occupied with the reconstruction of the infective bodies. Until this final maturation no serological specificity or infectivity is demonstrable and the virus is said to be in eclipse, although it is reproducing rapidly, of course.

The infectious body contains protein, and either RNA, DNA or both, and sometimes little else. Viruses are chemically austere probably because they are able to make such extensive use of the host's metabolism, including its synthesizing machinery, rather than simply because they are very primitive organisms. Some, in fact, seem to contain as much as 54 per cent of other materials, including lipids and even vitamins (Bauer, 1952). The lipid may serve as a cement between the protein and the nucleic acid (NA). It is, of course, difficult to obtain samples free of host material, and austerity of composition is probably the rule. The NA of the smaller viruses, which mainly infect plants, tends to be RNA, and that of the larger organisms, mostly attacking animals, to be DNA. The RNA viruses of insects usually grow in the host's cytoplasm and the DNA type in the nucleus, but this is far from being

general among viruses. Some nucleophilic animal viruses have only RNA while the bacteriophages have mainly DNA yet live in the host's cytoplasm (Burnet, 1960).

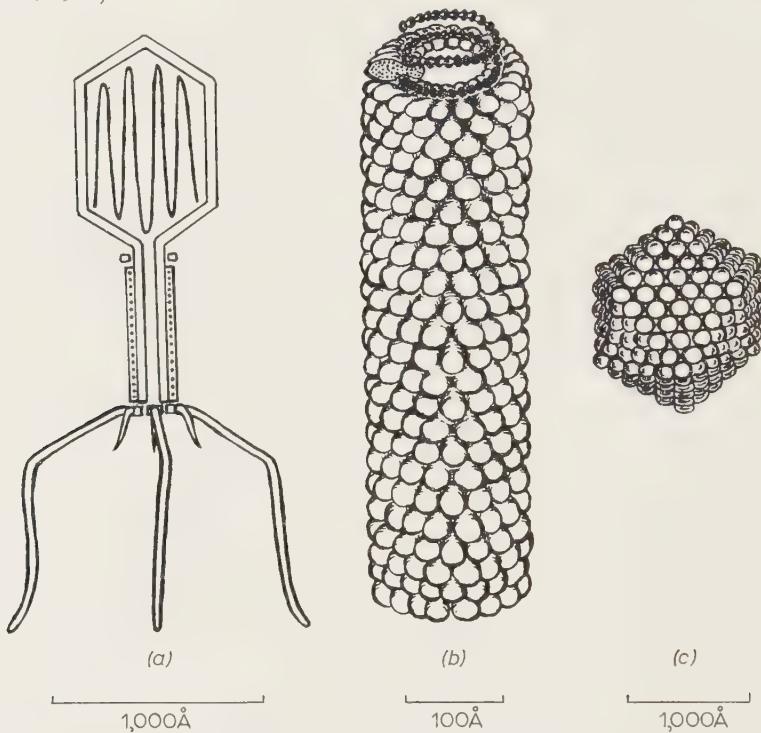


FIG. 12.1. DIAGRAMS TO ILLUSTRATE THE PROBABLE STRUCTURE OF THREE TYPES OF VIRUS

(a) The *T<sub>2</sub>* bacteriophage, one of the most highly differentiated. Its polyhedral head consists of a shell of protein containing a folded strand, or strands, of DNA. The tail is a hollow tube or "core" of protein, with an outer protein sheath and a separate collar. Basally are 4–6 short spines and 4–6 tail fibres which are usually extended, as shown, but can be wrapped spirally round the tail sheath. (b) Tobacco mosaic virus (TMV), a long hollow cylinder formed by a spirally wound chain of protein units, with a strand of nucleic acid sandwiched between the turns. This is represented as exposed at one end, by picking away the protein. (c) The icosahedron type, typical of the large polyhedral viruses of insects (1300 Å) but probably also of many others, ranging in size down to the minute  $\phi X-174$  bacteriophage (250 Å). The spheres represent protein units.

((a) Based on Kellenberger, 1961; (b) and (c) based on Klug and Caspar, 1960)

Even the amount of protein may be small, and in some types, at least, virtually none of this enters the host. It is simply a container for the more essential component, the NA, and it acts rather as an animated hypodermic syringe for injecting the latter into the host. It is not surprising, therefore, that little enzyme activity which could be considered specific to the virus can

be detected either in the virus material or in the infected host. Nevertheless some consider that enough protein enters the host to be significant (Pirie, 1960; Burton, 1960).

The typical bacteriophage enters its host, multiplies in the eclipse period, matures and then induces dissolution of the host. Other types release the mature progeny continuously, sometimes for as long as 100 hours or more (Dulbecco, 1957). The eclipse period includes a lag period and a variable amount of the following period of biosynthesis; it may be as short as the few minutes of the "burst" type or as long as two hours (poliomyelitis), or even days (Thomas, 1960). There has been considerable uncertainty whether the growth rate is arithmetic or geometric, linear or exponential, but it seems probable that in many cases it is typically geometric (Hershey, 1952; Glass, 1957), as in any well-fed organism. Mutations occur during the growth period (Luria, 1959) and give rise to clones of the mutant type; this shows that proliferation is multiplicative and it is likely also to be exponential in time, that is to say the generation time is not very variable. The subsequent reconstitution, or maturation, rate may be linear (Dulbecco, 1957). The pattern of growth may vary in different types of virus, and Commoner (1959) believes that tobacco mosaic virus, a rod-shaped organism (Fig. 12.1 (b)), grows in simple linear fashion. However, if it divides periodically and every daughter grows linearly, the increase in numbers will be geometric again.

The calcium ion promotes the adsorption of phage virus to the bacterial surface. Penetration of the host does not necessarily induce growth directly and in some viruses, at least (Huppert and Sanders, 1958), the triggering of synthesis in the host may be effected by quite non-specific, and even non-viral, material. Normally, however, the viral material no doubt does perform this function. The influenza virus appears to have an "antireceptor" enzyme, perhaps its only intrinsic enzyme, which destroys the host's surface receptors (Hirst, 1948) and so causes viral adhesion to its surface. The mere act of penetration is certainly a remarkable ability for such simple organisms. Rabies, verruca and others go on to penetrate the nucleus of the host cell.

Once inside the host the phage virus breaks down into the proliferative or reproductive bodies and, according to Hershey (1952), it then differs from the original infective body in everything except genetic continuity, certainly in all the properties which can be tested. Many other viruses probably break down in the same way (Burnet, 1960). However, some, the T<sub>2</sub> and T<sub>4</sub> bacteriophages, and those animal viruses with RNA as their main NA, remain a single unit, as tested by the effect on them of a single hit with ionizing rays (Dulbecco, 1957). The sub-units of the fragmenting type of phage are qualitatively incomplete virus material, yet capable of self-replication. The DNA of this type breaks into one large particle, approximately half the whole, and many small pieces; conceivably the former is a model for the eventual reconstruction of mature, genetically specific, virus.

The effect of a phage entering a bacterium is immediate and dramatic. The

host's own synthesis and division cease at once and it continues to live with its metabolism diverted to the synthesis of virus-type material. It enlarges as a result of this activity and the viruses of animal cells actually induce their proliferation, but in these cases the host's metabolism is not completely arrested (Burnet, 1960). The synthesis of protein continues unabated in infected bacteria, but the proportions of the various amino acids incorporated change, and a new, virus-type protein is produced. RNA production continues, at a low level, but the ratios of its nucleotide bases change to correspond with those of viral DNA (thymine being represented by uracil). The host, or at least the combined host-parasite system, now destroys any new invading individuals (Adams, 1959). In due course it also produces an enzyme, endolysin, which causes the host's dissolution. In animal cells the nucleolus enlarges and much more RNA is synthesized than in an infected bacterium. Some of this, at least, maintains the host's own synthetic activity. Tobacco mosaic virus varies to some extent in composition according to the host strain (Pirie, 1960), again indicating more influence by the host than in the case of bacteriophages.

Respiration rate changes little in infected bacteria but as in embryonic and cancer cells it becomes more anaerobic in type (Putnam, 1953). Such poisons as cyanide and dinitrophenol, therefore, can be used to kill the host and leave the virus (Adams, 1959). Here anaerobic respiration would seem to be related specifically to synthesis (p. 260) since there is no cell division.

In infected bacteria the whole of the new pattern of synthesis depends on the phage, and it is significant that the virus, in its host, will grow in a medium which permitted no growth of the uninfected host.  $T_2$  phage is able to restore to a "thymineless" strain of its host the lost ability to synthesize thymine. Four new enzymes, at least, appear, one for conjugating glucose, two for making the special hydroxymethylcytosine nucleotide of the virus and one for preventing cytosine itself from being incorporated instead. Penicillin and ultraviolet light inhibit the growth of the uninfected host but may have little effect on activities in one infected with the T-even phages (R. C. Williams, 1959).

The invading phage material, virtually pure DNA, is able to direct the protein-synthesizing activity of its host along its own lines, and this is the first stage of synthesis specific to the virus. By an ingenious method of selectively inactivating DNA, in the type of phage which is sensitive to this, Stent showed that the DNA is essential to initiate the synthesis of the specific protein (Glass, 1957). It probably does so through the intermediation of the new type of RNA already considered. If irradiation is delayed until the synthesis of the "early protein" is under way then this continues: either the protein is now autosynthetic, or it protects the DNA from serious damage, or full genetic information has been passed on to the new RNA, which is relatively insensitive to irradiation. An early protein component is synthesized also by other viruses (Thomas, 1960).

As yet no new DNA of virus type has been formed and this appears to depend for its synthesis in turn on the first-formed protein. Chloramphenicol

is a specific inhibitor of protein synthesis, and in its presence DNA synthesis also fails. The genetic information, therefore, appears to be passed on from nucleic acid to protein in a way which offers the best available support for the idea that the two components are synthesized by reciprocal heterotemplating (p. 204). In this event it is difficult to maintain that genetic specificity depends on nucleic acid alone.

Little of the first-formed protein is found in the mature virus and it is probably required purely for NA synthesis. However, it is no longer required once DNA synthesis is under way, an exact parallel to the previous and reciprocal situation during protein synthesis. It should be appreciated, of course, that radiations and chloramphenicol inhibit the *syntheses* of DNA and protein and have little effect on them as formed material. If the action of each on the synthesis of the other is catalytic or enzymic then quite a small amount once formed could continue as a potent agent for growth, though the rate of this should now be strictly linear and not exponential. There is evidence that the action is, in fact, catalytic: if more of the one is being synthesized then the rate of the other tends to become exponential: if not it remains linear. If the early protein is thus enzymic then it is not strictly true to say that the virus has no enzymes of its own.

The so-called *prophage* of some strains of phage is able to synthesize phage-type protein, presumably early protein, but not DNA subsequently (Lwoff, 1952), and so it remains non-infective, but yet transmissible down the generations of its host, since in this form it does not arrest the host's reproduction. It may be transformed into infective phage at any time by the administration of appropriate extraneous DNA, even some forms of foreign DNA. This is an awkward fact: it is possible that the prophage can modify foreign DNA to its own pattern even though it cannot synthesize the latter *ab initio*, but if so why can it not make similar use of the host's DNA, at any time? If some extraneous forms of DNA are effective without conversion to phage type then the unique genetic importance of the latter is suspect. Once activated to the infective state the phage uses host DNA, at least as a source of raw material, and it is clear that the prophage presents a number of intriguing problems.

The new DNA synthesized by active phage in turn catalyses the synthesis of a further generation of protein, the definitive protein for the eventual infective bodies. This protein will combine with the DNA for this purpose but the combination is not simultaneous with the synthesis of the protein (Thomas, 1960), as it appears to be in tobacco mosaic virus (Commoner, 1959); there, in fact, both components are synthesized, and conjugated, simultaneously (*but see* p. 159). Proflavin is a very specific inhibitor of the final, conjugation stage in bacteriophage.

These are the main contributions of the phage itself; some enzymes, particularly permeases and the enzymes not directly concerned in the synthesis of taxonomically specific materials, come from the host, along with some raw material. The rest of this is obtained from the medium, after infection, and is

processed largely by the persisting host enzymes. The relative contributions by enzymes of host pattern and those formed to virus specification, and the mode of cooperation between them are still uncertain, though it is likely that host systems play a greater part in those animal and plant cells which continue to grow and proliferate after infection.

In bacteria as much as 80 per cent of the raw materials, as measured by the elements C, N, and S, is obtained from the medium after infection, and this is perhaps the best evidence that much of the host's metabolism continues relatively normally. Nearly 20 per cent comes from the host's initial material so that the parent virus contributes less than 1 per cent. This is a powerful reminder that biological material is potentially common property, as the famous Yorkshire song more colourfully expresses it, and that taxonomic snobbery depends entirely on the way the material is put together! Moreover only about 70 per cent of the parent phage's DNA reaches its progeny (Thomas, 1960), presumably because of fortuitous losses, while most of its protein was abandoned at the outset. The progeny receive a higher percentage of their nucleic acid than of their protein from the host's initial material, conversely to what might have been expected from the order of their synthesis. This is, in fact, part of the evidence that the protein formed in the first period is not ultimately incorporated into the progeny. Nucleic acid and protein damaged by radiation can be used, so that it may be concluded that their components are still effective building stones. This and other facts show that the synthesis of virus-type protein and NA always proceeds via free a.a.s and nucleotides. Material from other viruses also can be used, even when these themselves survive and proliferate, as they do in cases where multiple infection is possible. Available materials therefore move with great freedom between the various taxonomically specific syntheses. If material of one virus were incorporated without change into another there would be little real distinction from true genetic recombination (see below) or hybridization.

The nutritive requirements of viruses are those of a typical heterotrophe, namely a.a.s, nucleotide bases and B-vitamins in particular, so that they are not grossly peculiar organisms. The bacterial and plant virus is of course less autotrophic than the intact host and new demands appear at infection. Like that of all parasites viral anabolism is deficient to a degree and even animal viruses may be more demanding than their host cells. Some changes are more obscure: DNA-synthesis changes in character and unlike that of the intact host is in some cases insensitive to irradiation. Reciprocally RNA synthesis may become sensitive, in contrast to the behaviour of the system of the intact host (Putnam, 1953).

The growth cycle of viruses attacking plant and animal cells is not so well known as that of the bacteriophages, partly because it does not dominate that of the host cell, and certainly not that of the whole body, as much as phage activity. The whole tempo of growth is slower and this may be the main reason for the long eclipse period, and the progressive rather than simultaneous

maturity of infective bodies. There is evidence that the invading particle breaks down (Burnet, 1960; Thomas, 1960), that only its NA is subsequently active, and that several sequentially or simultaneously formed components eventually combine to form a mature infective body. The components are protein or soluble ribonucleoprotein, each alone without antigenic or infective power. There appears to be the same alternating sequence of protein and NA synthesis in some (Schramm, 1961) as in bacteriophages. Tobacco mosaic virus at first forms large amounts of RNA and no protein, while later protein synthesis is 2,000 times greater than that of RNA. The DNA of the host's nucleus appears to be extensively involved in the early stages of synthesis of both NA and protein of most of the small viruses, but is not used by the virus of smallpox, which is a large virus. It has a large DNA particle of its own (Burnet, 1960), which does not fragment. Consequently it is also more easily recognized and traced than that of the smaller viruses. In some cases the host's cytoplasm provides a coat round the completed virus (Burnet, *i.c.*); in general activity is more dependent on the host than in bacteria. Even among bacteriophages the degree of dependence on the host is very variable (R. C. Williams, 1959).

The pattern of synthesis in animal viruses is very similar to that of the host's own biosynthesis: the initial activity is in the nucleus, virus specific nucleoprotein emerges and synthesis then continues in the cytoplasm. In the large psittacosis virus binary fission is thought to occur (Luria, 1959), and there may be no sharp distinction between viruses and larger parasites which infect the cells of animals and plants.

Perhaps the most enigmatic feature of the growth cycle of viruses in general is the initial fragmentation for purposes of synthesis and the late reconstruction, which in some respects may be compared to differentiation in higher organisms. The main significance of the fragmentation may be to facilitate genetic recombination. The reconstruction is a good example (*p. 7*) of synthesis in the sense of building larger units, but with a loss rather than a gain in total free energy of the material concerned—unless, of course, the host supplies the energy for conjugating the components.

The genetic processes in the cycle are most interesting when viruses of two or more strains of the same species grow in the same host cell. The mature bodies then emerging may have some of the genetic properties of two, or even all three, parental strains. There is now some knowledge of gene sequences in the genomes of some viruses and the hybrids are found to have sequences from the different parental strains. The fragments of each initial infective body replicate as fragments, and "partial replicas" from different strains can unite to form hybrid proliferative bodies. Lederberg favours the alternative of "copy choice" (Thomas, 1960), that is selective copying of the successive stretches of the complete genome from appropriate stretches of the different parental genomes. In either case the hybrids may then replicate as hybrids. Hybrids containing genetic material from all three parents are too frequent for the phenomenon to be in the nature of orthodox crossing over, which would have

to proceed in steps, each involving only two parents. Occasionally hybrids are heterozygous for a piece of a linkage group, but usually they are pure and, therefore, presumably haploid, though some of the animal viruses seem to be highly polyploid (Burnet, 1960). It might seem that haploid hybrids would have little chance of segregating the pure original strains but in fact some such segregation does occur (R. C. Williams, 1959); if hosts are infected with the experimentally mixed nucleic acids of two parental strains some lesions with mixed properties result, but these later yield pure strains again. If the hybridizing fragments are relatively large fractions of the whole genome then this might occur fairly frequently by chance alone. Hybridization occurs before the final conjugation between NA and protein but there also occurs what is called "phenotypic mixing" (Luria, 1959), the simple association of material from different strains during the final reconstruction. The protein of one strain may become wrapped round the NA of another; this union does not seem to be as genetically specific as might have been anticipated, therefore.

These genetic phenomena illustrate once again the great plasticity and versatility of viruses, and this is shown also by experimental work in which hybrids of an RNA type of virus are formed by mixing the NA of one strain with the protein of another (Fraenkel-Conrat *et al.*, 1957; Wang and Commoner, 1956); this pseudo-hybrid acts as an active infective virus. Its infectivity, that is the ability to grow in a host, depends mainly on the RNA component and is abolished by ribonuclease. However, it is also destroyed by antibodies against the protein component, and neither component alone is adequate (Brachet, 1957; Commoner, 1959; R. C. Williams, 1959). This seems to imply that in such cases the entry of a certain amount of virus protein into the host is indispensable. On the other hand the decisive importance of the NA component is again emphasized by the fact that in the course of time the protein of an artificial hybrid of this type is replaced by that typical of the species donating the nucleic acid. The precise genetic significance of the two components is still somewhat uncertain (Commoner, 1959).

The malaria parasite and certain others belonging to a size range and structural rank above that of the viruses are nevertheless intracellular parasites. They behave like the viruses in a number of respects relevant to growth (Trager, 1960), causing cell hypertrophy and sometimes hyperplasia. They also have an eclipse period, and they modify the metabolism of the host's cell in specific ways (McKee, 1951).

It has been pointed out (Haldane, 1954) that the nucleus of a spermatozoon uses the foreign cytoplasm of the egg in much the same way as a virus does its host's cytoplasm and there are other analogues in the melanogenic particles of Medawar (1947) and probably in carcinogenic cytoplasmic particles (p. 102), the kappa particles of *Paramecium* (p. 143) and others. The normal cell may be regarded as a system of cell components selected for harmony in their growth and general activity; there is nothing extraordinary, therefore, in the natural selection of a foreign subcellular system which can harmoniously modify the

cell for the production of its own specific material. It is significant that the virus is usually highly specific to its host species and even to one particular tissue. Harmless forms of virus such as the prophage (p. 157) are perpetuated indefinitely, attached to the genome of their host, and there may be symbiotic forms similarly perpetuated, and having a permanent representation in the genome. Reciprocally pieces of the genome of the host sometimes become attached to that of the virus, which then *transduces* them into a new host; there they multiply at the same rate as the carrier virus (Thomas, 1960). Such foreign components might be indistinguishable from the host's own materials by all available tests, and the possibility of permanent evolution of this type has been envisaged (Darlington and Mather, 1949). Detached pieces of the bacterial genome ("episomes") are virtually indistinguishable from viruses.

The multiplication of viruses provides us with a glimpse of the critical, taxonomically specific level of biosynthesis of the two most indispensable constituents of living matter, NA and protein. As Bawden and Pirie (1952) remarked, the multiplication of viruses is likely to teach us more about protein synthesis than *vice versa*. It also illustrates inheritance, or the transmission of genetic information, in a state so simple that it is almost a growth phenomenon itself. There appears to be an act of mating every time a T-even phage duplicates (R. C. Williams, 1959). Crick (1961) and his colleagues, in fact, are using the genetical analysis of phage virus in a direct attempt to decipher the code which determines the synthesis of specific protein molecules (p. 205).

## CHAPTER 13

### *The Growth of Skeletal and Other Materials*

As indicated at the end of Chapter 11, there is a group of materials the synthesis of which begins in the size range between that of the standard cell organelles and that of peptide molecules, which are the ultimate building bricks of the living fabric. These materials are pure and mixed polymers of peptides, often together with other material, both organic and inorganic. The majority, and certainly the bulk, of such materials are used to build skeletons and they have been the most studied, because of the practical advantage of having large quantities to handle. The contractile material of muscle cells also may be included, together with other cell-specific proteins, and blood fibrin. One of the important features of all these materials is that although the essential step in their growth is the simple polymerization of molecules to form macromolecules or micelles, there is no upper size limit and many of them manifest growth at all levels from the electron microscopic to the gross macroscopic. The skeleton of a blue whale weighs many tons (p. 11) and that of corals, collectively, has formed reefs a thousand feet deep or more, along many thousands of miles of tropical coastline. The growth of a vertebrate is largely determined by that of its skeleton, and in general these materials represent a component of growth which is of major importance qualitatively as well as quantitatively.

The more massive skeletons are eventually deposited extracellularly, and often extra-corporeally, and probably owe their large size mainly to this. Most skeletal material in fact is ultimately extracellular, exceptions being notochord material and the keratin of vertebrate epidermal skeletons. Although many skeletons are highly mineralized, there is always a protein component and it usually forms at least the primordium of each skeletal unit. It usually controls the deposition of the other components (Irving, 1957), so that this chapter is primarily a study of the growth of protein macromolecules and of their effect on the growth of other components. Some knowledge of the composition and final structure of the materials is first necessary.

As much as 98 per cent of the enamel of teeth and of siliceous sponge spicules is mineral but usually there is more organic matter and this is as much as 30 per cent even in bone, so that when demineralized it retains its coherence and shape. Three types of mineral are common in animal skeletons. A hydrated silica,  $H_2Si_3O_7$ , occurs in the spicules of some sponges, in the skeletons of many Radiolaria (Fig. 13.1), and of some Testacea, and more sporadically elsewhere: for instance there is silica in feathers. Calcium carbonate is the most common

type of mineral matter, occurring in the shells of Foraminifera, the spicules of the Calcarea, the skeletons of many Cnidaria and of the echinoderms, the shells of molluscs and brachiopods, the exoskeleton of Crustacea, millipedes and some Polyzoa, the tubes of serpulids and elsewhere. The egg shell of birds and reptiles, and of some snails and other animals is mainly  $\text{CaCO}_3$  and

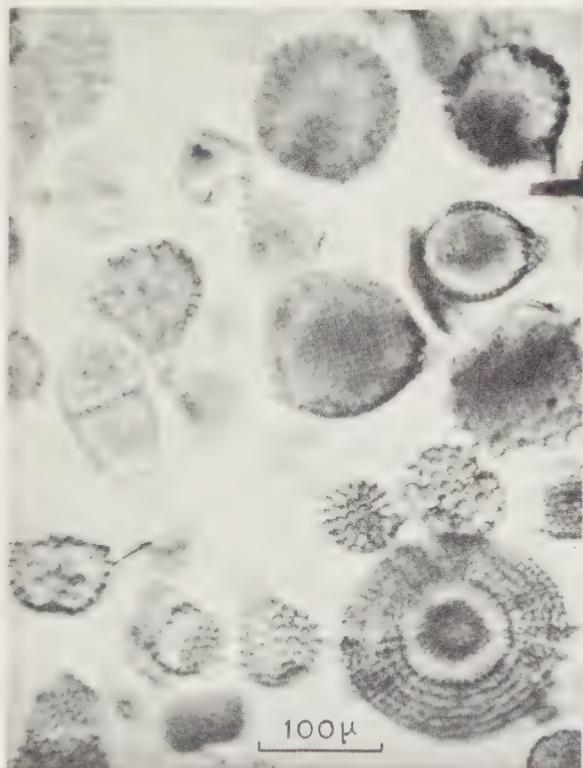


FIG. 13.1. SKELETONS OF TYPICAL RADIOLARIA TO SHOW PERFORATE MASSIVE FORM AND GEOMETRIC REGULARITY

(Photo: J. S. Haywood)

its formation is instructive here. The third main type of mineral is hydroxyapatite,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ , peculiar to the vertebrate materials bone, dentine, and enamel, though as much as 17 per cent of the mineral of crustacean exoskeletons is phosphate, and it is the major constituent of the shell of some brachiopods. There are considerable amounts of other anions and cations in bone material, particularly  $\text{CaCO}_3$ , and gross contaminants are usual also in other skeletons. There is 7 per cent of  $\text{MgCO}_3$  in calcareous sponge spicules and as much as 17 per cent in crustacean skeletons. Contaminant iron salts give the bright red colours to the skeletons of some Alcyonaria. Some Radiolaria have skeletons mainly of strontium sulphate, and other materials are occasionally

major constituents; like the various contaminants these only serve to emphasize the extent to which a few main materials have been exploited.

The minerals are usually crystalline, though the 10 per cent of  $\text{CaCO}_3$  in bone, and certain others, are amorphous. Crystalline  $\text{CaCO}_3$  is usually calcite in cooler waters and aragonite in warmer latitudes, though evidently this is not *de rigueur* since some molluscs have both, even in the same shell! The Crustacea have vaterite and not aragonite as their second crystalline form. Mineral in general makes skeletons rigid and hard wearing, though brittle in the absence of adequate organic matter.

In most of these materials the texture is all-important. The main proteins of skeletons, and of the other materials under consideration are fibrous, giving them coherence, pliability, and strength. These proteins can often be broken down to molecular or macromolecular units, which may also be the original building bricks since they readily repolymerize under appropriate conditions. Sodium sulphide breaks down the keratin of hair and epidermis to molecules  $170 \times 11 \text{ \AA}$  (Matoltsy, 1958). Collagen is resolved into, and probably built up from, tropocollagen units of about this same diameter, but  $2,600 \text{ \AA}$  long, consisting of three helical peptide chains (Fig. 14.1) plectonemically co-coiled (Picken, 1960, p. 384). A stouter unit, about  $30 \text{ \AA}$  in diameter, the *protofibril*, is more commonly the limit of resolution. It contains up to ten peptide chains, lying parallel or plectonemically twisted together (Fig. 11.1). It is often possible to recognize other definite size stages in the growth of protein fabrics, namely the *filament*, about  $100 \text{ \AA}$  thick, the *fibril* up to  $1,000 \text{ \AA}$  in diameter, and finally the *fibre* which may be visible to the naked eye. However, the diameters of these units vary considerably between the different proteins and taxonomic species so that collectively, at least, there is almost a complete spectrum of intermediate sizes.

There is often a parallel alignment of units, at all levels of size, and this is best seen in the collagen fibres of tendons. At the other extreme the fibres, or bundles of them, may interlace in all directions, as in the general connective tissue and in a blood clot. In hair only 10 per cent or so of the keratin is fully orientated along its axis. In the capsules of many organs, in the walls of blood vessels, and in bone, collagen fibres are arranged in layers, or lamellae, all parallel within one layer and making a large angle with those of the adjoining layers. This ply structure is seen also in the test of tunicates and in the arthropod skeleton (Fig. 13.2), and gives maximal strength against forces in all directions. The other arrangements found also usually prove ideal for their particular functions. In the epidermal cells, subject to forces in various directions, the keratin fibres lie randomly in the plane of the skin but in the main layers of the hair (Fig. 4.23) they all lie more regularly parallel to its axis. The proximate cause of each variant, as a growth phenomenon, is usually more obscure, however.

Structure is often orderly along the length of the fibres also, and the units are often so precisely in dress that heterogeneities appear as distinct transverse

bands under the microscope or the electron microscope. The muscle sarcomere varies in length up to  $1\text{ }\mu$ , while the natural period of mature collagen fibres is  $640\text{ \AA}$  and that of the developing fibres about one-third of this. These are



FIG. 13.2. VERTICAL SECTION THROUGH THE EXOSKELETON AND UNDERLYING TISSUES OF THE LIMB OF THE CRAYFISH, *Potamobius* (*Astacus*)

The section is cut along the limb, through the preformed autotomy plane, near the base. *AM*, connective tissue membrane across the limb, just proximally to autotomy plane; *CT*, connective tissue; *Ed*, epidermis; *En*, endocuticle; *Ep*, epicuticle; *Ex*, exocuticle; *F*, fissure through exoskeleton at autotomy plane; *S*, seta (base); *SE*, specialized epidermis at the autotomy plane.

(Photo: J. S. Haywood)

thought to be due to the tropocollagen units, appropriately staggered. In the laboratory other periodicities can be produced by repolymerization of the degraded native protein. Collagen often gives a "long spacing" period of

2,000 to 3,000 Å, the full length of the tropocollagen units, while muscle paramyosin can form a short one of 400 Å.

Cellulose fibres, formed by simple linear polymerization of  $\beta$ -glucose units, are the main skeletal elements of the test of tunicates and they are contributory fibres in the connective tissue of vertebrates, particularly in certain pathological conditions. In animals they are associated with protein fibres of the collagen type. In *tunicin* they form regular layers of parallel fibres, the orientation varying 90° between successive layers as in the cell wall of plants (Picken, 1960, p. 430).

In the arthropod exoskeleton the main protein, arthropodin, is interstranded with another polysaccharide derivative, chitin, which is a linear polymer of acetyl- $\beta$ -glucosamine,  $\text{CH}_2\text{OH}\cdot[\text{CHOH}]_3\cdot(\text{CH}_3\text{CONH})\text{CH}\cdot\text{CHO}$ . The association is probably a true chemical union formed at or near the molecular level, in stoichiometric proportions, since the resulting protein-polysaccharide complex can be dissolved and reprecipitated repeatedly without separation of the components (Richards, 1951, p. 82). The repeat period of chitin is 10.3 Å, and is due to the chitobiose unit consisting of two acetyl glucosamine residues; sterically this would fit quite closely the span of three amino acids in peptide linkage (Bonner, 1952, p. 44). Chitin stabilizes the protein and decreases its solubility; it also contributes tensile strength but does not harden it significantly.

Hardening of the arthropod exoskeleton is achieved primarily by "tanning" the protein, that is by binding its molecules together with phenolic substances, oxidized to quinones in the process (Pryor, 1940; Richards, 1951). The resulting material, sclerotin, has remarkable tensile strength and at the same time is very pliable. This pliability would be a liability in the larger Crustacea, the Xiphosura and some other arthropods, where great strength against compression also is necessary, and these arthropods have, therefore, added minerals, increasing rigidity at the expense of pliability, which remains only at the joints and in other special places. The keratin of vertebrates has even greater strength and pliability than the insect exoskeleton, due mainly to the disulphur bonds between the protein molecules. In bulk keratin also has considerable rigidity and strength against bending, for instance in nails, claws, and horns. Quill feathers have a high degree of rigidity in spite of their relative slenderness, and this may be due mainly to their high silica content.

Keratin fibres are embedded in a more amorphous matrix of a second protein, keratohyalin and such matrices are rather common in the materials under consideration. As in this case, they often contain material similar to the fibrous component, but with its molecules more randomly arranged. This increases the strength against forces in all directions and at the same time a matrix strengthens the orientated fibres themselves by holding them in place and by cushioning them against damage. Matrices vary considerably in composition and in consequent effect on the properties of the material as a whole.

In the connective tissue of vertebrates and probably of most animals, the

collagen fibres are embedded in a matrix of mucopolysaccharides, molecules of protein conjugated with polysaccharide. Part of this may be fibrous and the rest completely amorphous. From it a polymer known as hyaluronic acid may be obtained (Picken 1960, p. 388), consisting of equimolar amounts of acetyl glucosamine and another hexose derivative, a glucuronic acid. To some extent the chitin of arthropod exoskeletons may be compared with this matrix, therefore. In life the hexosamine is probably esterified with sulphuric acid and the matrix of cartilage contains a polymer of sulphonated acetyl- $\beta$ -galactosamine,  $\text{HSO}_3 \cdot \text{CH}_2\text{O} \cdot [\text{CHOH}]_3 \cdot (\text{CH}_3\text{CONH})\text{CH} \cdot \text{CHO}$ , again with an equal amount of glucuronic acid  $\text{HOOC} \cdot [\text{CHOH}]_4 \cdot \text{CHO}$ . This polymer is known as chondroitin sulphuric acid. Cartilage is outstanding for the very high ratio of matrix to fibres and the resulting material has much the properties of a hard rubber such as gutta percha. There are smaller quantities of chondroitin sulphuric acid also in the matrices of connective tissues and in tendon and bone.

There is considerable similarity in composition between the various skeletons, even between such diverse examples as vertebrate bone and arthropod exoskeleton. These both have the main toughened protein fibre, the matrix of protein and polysaccharide, and the hardening mineral.

Bone	Crustacean Exoskeleton
Osseomucoid	Chitin
{ Chondroitin Protein	Arthropodin
Collagen	Sclerotin
Apatite: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$	$\text{CaCO}_3$ and $\text{Ca}_3(\text{PO}_4)_2$

The mineral components of skeletons also show considerable orientation. The spicules of apatite in bone lie parallel to, and even within, the collagen fibres (Glimcher, 1959). They therefore also lie all parallel within one lamella and at a large angle to those of adjacent lamellae; this gives great additional strength against forces in all directions. The crystallites which make up the spicule, however, are preferentially orientated not along its axis, but either in the axis of the whole bone or at right angles to this, circumferentially (Clark and Iball, 1954). At least two independent formative factors must be involved, therefore, and the spicule is not a natural crystal form.

A similar phenomenon is seen in calcareous sponge spicules (Jones, 1954; 1955). The triradiate spicules of *Leucosolenia* are very regularly orientated relative to the tubular body of the sponge (Fig. 13.3) and vary in shape systematically along the tube, the *oscular angle* between the two distalward-pointing rays being maximal near the osculum itself. The optical axis of the crystallites of  $\text{CaCO}_3$ , however, is not parallel to, and in fact bears no unique relation to, the morphological axes of the spicule. It is parallel throughout all the rays of one spicule, which, therefore, appears as though carved out of a

single large crystal without attention to its optical axis. If spicules are placed in a suitable solution *in vitro* calcite continues to deposit on them with the same orientation, so that, once determined, the optical axis is probably maintained by the forces of simple crystallization. While the optical axis rarely coincides

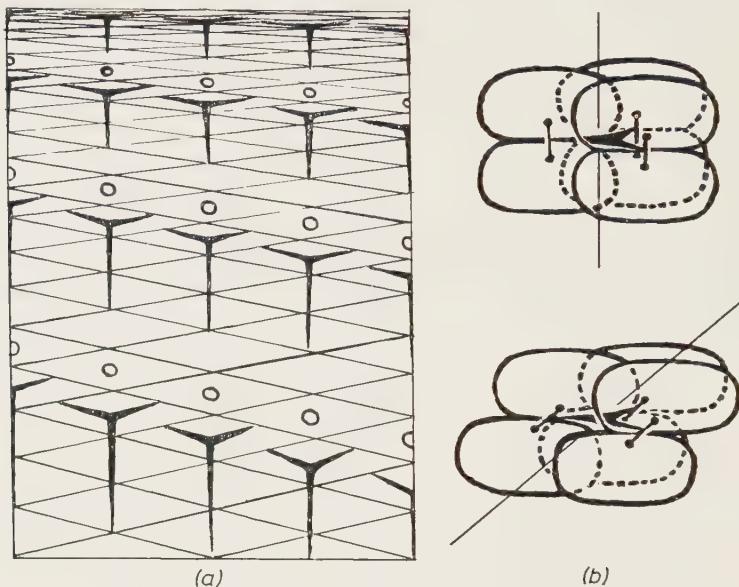


FIG. 13.3. (a) ARRANGEMENT OF THE TRIRADIATE CALCITE SPICULES IN THE WALL OF THE OPENED-OUT TUBE OF THE SPONGE, *Leucosolenia*

Around the osculum, at the top, the two oscular rays enclose an angle of nearly  $180^\circ$ ; this angle decreases progressively towards the base of the tube, possibly determined by a progressively changing orientation of fibres in the mesogloea, represented by the superimposed mullions.

(b) DIAGRAMS SHOWING THE MODE OF ORIGIN OF THE TRIRADIATE SPICULE OF *Leucosolenia*, AND JONES'S VIEWS ON THE MODE OF DETERMINATION OF THE OPTICAL AXIS OF THE CALCITE CRYSTALLITES, INDICATED BY THE RULED LINE

The spicule arises in the plane between the two tiers of three cells, which is also the plane of the wall of the sponge. Each ray is formed between a pair of sister cells. The orientation of the calcite crystallites may be parallel to the division spindles (dumb-bell symbol) which gave rise to the pairs of cells. In the simplest case (*above*) this is normal to the plane of the morphological axes, but shearing forces may tilt the division spindles (*below*) and the optic axis.

with the morphological axis of any ray it usually is in the same *plane* as the basal ray and so also in a longitudinal plane bisecting the tube of the sponge. However, the "optical angle" between the optical axis and that of the basal ray also varies in a systematic way along the sponge, independently of that in the oscular angle. Thus, not only is it independent of the morphological axes of the spicule, as in bone, but both vary systematically along the body; both features present challenging problems in growth.

Jones has suggested an explanation of the systematic variation, based on the idea of graded mechanical properties in the mesogloea and in the two cell layers of the sponge. The difference between the optical and morphological axes of a spicule he suggests may be due to shearing forces during cell division, displacing one tier of three formative cells relative to the tier of sister cells (Fig. 13.3 (b)), the division spindles between sister cells determining the optical axis, if not actually forming the primordium of the spicule. In echinoderm spicules (Schmidt, 1932) the optical axis in fact is regularly perpendicular to the plane of the spicule, which as in the Calcarea begins as a triradiate. The shearing force and the resulting shear are thought to vary systematically along the body of *Leucosolenia*.

The morphological axes of the calcareous sponge spicule, on the other hand, are thought to be determined by the positions of the three pairs of formative cells, but this only advances the solution one step. As in the case of the organic fibres (p. 164), the morphogenetic determination is obscure, though the biological value of the results is evident enough. Sponge spicules fit the anatomy and histology of the animal very well (Thompson, 1942), and most other mineral components are constructed for functional efficiency, as already shown for the bone spicules. The result is often also aesthetically pleasing (Fig. 13.3), and the beauty of radiolarian skeletons (Fig. 13.1) and holothurian spicules (Fig. 13.7) may be more than incidental. In some axiferan Alcyonaria alternate sections of the organic axis are calcified, leaving useful flexible joints between. The massive character of many mineral skeletons undoubtedly also is adaptive: many of them begin as isolated spicules or spherules but later become massive in various ways.

It seems clear enough that the fibrous protein is indeed the key component of most of these skeletal and other materials and that its growth should be considered first. The initial stages of synthesis, usually extending up to the initial molecular polymerizations of peptides, are intracellular (Mercer, 1958) but in many cases the later stages are extracellular, and often proceed without enzymic help, with ready spontaneity. Cells which produce protein for deposition extracellularly have a well-developed endoplasmic reticulum (p. 146) and fibres or tubules of protein are sometimes seen in process of extrusion, in cells of silk glands and in fibrocytes. By contrast, epidermal cells, which retain their keratin, have numerous ribosomes but few membranes.

A good deal of light has been thrown on the later stages by laboratory studies on the fresh precursor protein or on the products of degraded fibres. Collagen is readily soluble in acetic acid and can be reprecipitated as banded fibres with the natural period of 640 Å, simply by raising the pH or adding salts (Gustavson, 1956). Paramyosin, the main protein of many lamellibranch muscles, likewise dissolves in acetic acid and re-forms threads when the ionic concentration is increased (Schmidt, 1955). The conjugation of the two main proteins of skeletal muscle, actin and myosin, is essentially a process of macro-molecular growth; *in vitro*, and no doubt also *in vivo*, it is similarly sensitive

to changes in ionic concentration, and is readily reversible by this and other means. This is at the same time the best instance (p. 5) of a work function based on a reversible synthesis. The complete contraction cycle *in vivo* probably also involves the reversible polymerization of globular, G-actin to the fibrous, F-form. Myosin acts as a catalyst for this step, so that the whole process has a high degree of automaticity. It is also highly labile, moving either way under slight, physiological changes in conditions. This is equally true for the synthesis of most of the other proteins under consideration.

Fibrin formation occurs spontaneously on increasing the concentration of the soluble precursor, fibrinogen itself (Ferry, 1952). Although extracellular throughout, it proceeds in two stages which may correspond to the intra- and extra-cellular phases of other proteins. The first involves the union of a small number of monomer units of fibrinogen, always around 15, and is reversible simply by diluting the solution once more, although it is exergonic (p. 7) to the extent of 8,000 calories per mole of monomer. Moreover, although this first stage is said to be a state of minimal free energy and maximal entropy, the second stage proceeds spontaneously on further increasing the concentration to 0·5 grammes per litre. Soap micelles behave very similarly when they are concentrated, so that the spontaneity is not peculiar to protein macromolecules. The fibrinogen units are negatively charged and so repel each other, but once brought within a certain range, by concentration, this is outweighed by the local attractive forces of London and van der Waals. Polymerization occurs only between pH 5 and 9, which has been taken to imply that histidine or phosphate ester groups are involved, since they have *pK* values within that range. The fibrin eventually formed is stabilized by calcium (MacFarlane, 1956) a fact no doubt also relevant to mineralized protein skeletons. Fibrin has the power of slow contractility and belongs to the same general group of proteins as myosin (Astbury, 1945); it is noteworthy that calcium plays a part in the contraction cycle of muscle.

The behaviour of the small peptide, cofibrin, which normally keeps fibrinogen inactive, is of interest as a growth phenomenon. It is split off in the process of activating fibrinogen so that in this instance a minor molecular degradation permits a vastly greater subsequent synthesis.

It might be anticipated that protein monomers would polymerize either by side-to-side or by end-to-end linkages, or both, and, in fact, all three types have been recognized in biological materials. Fibrin formation (Ferry, 1952) is probably an example of the first and also the linkage between actin and myosin, while the polymerization of actin itself is an example of the second. *In vitro*, insulin polymerizes in a way similar to that of actin. Collagen is the best example of the third type, which no doubt gives the strongest texture, since protein molecules present many groups capable of forming H-bonds and other links laterally, while the terminal links may have the strength of peptide bonds. Both lateral and end-to-end linkages build fabrics with mainly parallel orientation of units, at all levels of magnitude, and this is the most

useful texture. However, if a unit is linked laterally to different neighbours at its two ends then a meshwork results, similar to that found at a higher level of magnitude in the vertebrate myocardium. If the only links are sparse lateral ones then a random feltwork is produced as in fibrin. Elastin has a larger number of bonds, more regularly arranged but it becomes birefringent, that is its constituent fibrils become parallel, only when it is stretched (Picken, 1960; p. 397); this is in keeping with its rubber-like properties. At rest it may have a rather regular structure of crossing systems of fibres.

It would also be anticipated that only fibrous proteins could undergo polymerization in this way but, in fact, G-actin and insulin are globular proteins and they produce fibrous polymers which may be compared to a string of beads. The peptide chain of the globular monomer does not first unravel as might have been expected and it may not be correct to describe the linkage as being end-to-end, though this is still uncertain. Any compact unit would be classed as a globule on most available criteria and a number of the polymers in group two may have genuine end-to-end linkages, i.e. between the ends of cylindrical bundles of molecules, rather than between points on the surfaces of globules; the latter would be in effect lateral bonds of the peptide chains. The monomers of keratin and collagen described above are, indeed, short cylinders of cabled threads. The potential energy barrier of forming links is minimal at the ends of the molecules (Picken, 1960, p. 246) and this is likely to be exploited in practice. Mercer (1958) suggests that the second group is actually the most common *in vivo*, so that the question of its mode of linkage is one of importance. The resulting fibres are easily recognized by their very uniform diameter and their great lengths, with few branches.

It seems probable that in this type most of the chemical groups capable of forming lateral bonds must be already satisfied between the constituent molecules and protofibrils and that this may also set the limit to the size of cable which can be built by successive grades of co-coiling. Very large cables are rare in living organisms, but it is not yet certain whether this is due to such mechanical restrictions on growth rather than to biological expediency. The texture of skeletal muscle and some other structures possibly may approximate to an array of microcables, and co-coiling up to the third order is indicated for some protein materials (Pauling and Corey, 1953). More open textures seem to be quite general, however, the individual filaments and fibrils having enough groups free to make occasional lateral bonds with their neighbours on all sides.

The formation of polymers with a parallel arrangement of their subunits is facilitated by the prior orientation of these. There are a number of natural forces capable of effecting this, but at present little evidence to show which is actually used (Picken, 1960, p. 251). So long as the units remain in suspension they may well be rotated into alignment by weak universal forces such as those of van der Waals (Randall, 1954). *In vitro* the collagen micelle can remain in suspension until it reaches a size of 4,000  $\times$  400 Å, but *in vivo* precipitation is

accelerated by the ground substance and so probably occurs at a smaller size. After precipitation the progressive accretion of small soluble units is the only mode feasible (Gerber and Altman, 1961) and ensures that the initial orientation is sustained subsequently. The discontinuity of size classes in some cases (p. 164) does not appear to depend on a geometric progression in the mode of polymerization, therefore.

The mass-action phenomenon is probably sufficiently operative at this level for precipitation of the polymerized products to favour further synthesis and it seems that the ground substance promotes collagen formation in this way. Very significantly it precipitates collagen most effectively at the relative concentration found in connective tissue, about 1/1,000 of the collagen concentration. Natural collagen formation is accompanied by increased metachromatic staining of the tissue, indicating that the acid polysaccharide component of the ground substance is increasing, and in fact chondroitin sulphate alone, without the protein of the ground substance, is effective in precipitating collagen in the presence of acetate (Jackson and Randall, 1953). Chondroitin sulphate also stabilizes the deposited collagen, just as chitin does arthropodin (p. 166). In the latter case the precipitating action is reciprocal and chitin is rarely, if ever, deposited alone. In general it seems that all components of each system of skeletal materials interact to promote the formation of the skeleton, as well as cooperating in its mechanical function once formed; further evidence of this is given below. It is probable that the collagen forms an actual compound with the mucoprotein of the ground substance and cannot be separated from this without changes in its properties (Picken, 1960, p. 389).

Although the synthesis of these materials is readily induced by relatively simple conditions *in vitro*, and therefore is likely to be relatively spontaneous also *in vivo*, it is certainly speeded by the universal energy mediator, ATP (p. 318). The process is not completely spontaneous, of course, but it is surprising that the high energy available in the pyrophosphate bonds of ATP should be necessary. In muscle-contraction, by contrast, the activity is cyclically repeated and obviously the high energy supply is necessary at one phase of every cycle. *In vitro* ATP induces the formation of fibres of paramyosin, actomyosin and collagen from their solutions (Hayashi, 1953; Randall, 1954), all with the characteristic banded structure. It also promotes the polymerization of actin and the conjugation of actin and myosin (D. M. Needham, 1952). Actomyosin is resolved into its two components again on further increasing the ATP concentration; this is the basis of its so-called plasticizer action in muscle relaxation. It is a further illustration of the great lability of synthesis at this level. Alkaline phosphatase is abundant in fibrocytes and is always very active in regions where fibrous proteins are being synthesized (Danielli, 1951): this may be connected more or less directly with the activity of ATP, since the enzyme can act as an ATPase and at any rate releases P from organic combination, which originally depended on ATP. The action of phosphatase on protein synthesis is evident even in skeletal materials which lack phosphate and

other minerals, and this is to be distinguished from its role in phosphate deposition (p. 178).

Among other factors significant in the synthesis of a number of these proteins there is a high concentration of tyrosine and a low concentration of the simple aliphatic amino acids, serine, and hydroxyproline (Bowes *et al.*, 1953). Tyrosine is abundant in the ground substance of connective tissue and promotes collagen formation; it is very curious, therefore, that gelatin, the main protein derived from collagen, yields much alanine and hydroxyproline and no tyrosine. By contrast, however, spongin, a rather collagen-like skeletal protein, and also the related gorgonin of axiferan coelenterates, contain considerable amounts of diiodo- and dibromo-tyrosine, while fibrin contains an unusually high percentage of tryptophan. These are probably good instances of the function of the aromatic amino acids in differentiative growth (p. 288), that is in the production of specialized proteins. The role of polyphenols in hardening the skeletal proteins of arthropods and many other animals is another instance, since the polyphenols are derived from these amino acids, ultimately. Elastoidin, and some other vertebrate materials related to collagen, also are hardened by polyphenols (Picken, 1960).

It seems reasonable to distinguish the group of proteins which are hardened and stabilized in this way from the keratin-myosin-fibrin group stabilized by disulphur, —S—S—, bonds. SH-groups, which oxidize and link in pairs to form the disulphur bond, appear to be used in the lateral adhesions of fibrin, and in the changes of muscle proteins during the contraction cycle, whereas in collagen, spongin, and gorgonin the sulphur content is conspicuously low. Both systems of hardening involve exergonic, oxidation reactions. It is interesting that the epicuticle of arthropods is often hardened by disulphur bonds and not by quinones (Picken, 1960), so that few of these processes have absolute taxonomic restrictions.

Arthropod chitin may be conjugated with protein before deposition in the exoskeleton and it may be transported in the blood in this form (Picken, 1960, p. 421). As already indicated (p. 166) the conjugate is very insoluble but since it can be dissolved from existing exoskeleton by the moulting fluid it may be carried in solution in the first place, though there is usually also a local storage of material in the epidermal cells. The formation of the chitin itself seems to be a unitary process, from the glucose monomers upwards (p. 234), and this may be true of the unbranched polysaccharides in general.

In a sense the deposition of every component of the exoskeleton of arthropods is part of its growth, but so many are actually formed elsewhere that assembly is a transport phenomenon rather than one of biosynthesis. The lipid and other materials of the skeleton depend on synthesis at the molecular level (Chapter 15). For other details the reader is referred to Richards (1951), Wigglesworth (1953, 1959), Dennell (1958, 1960), and Picken (1960).

The building of the mineral components of skeletons presents its own features of interest. There are two main methods of depositing the mineral,

depending on whether the skeleton is internal or external to the body. In the internal skeleton a fibrous protein is first deposited and orientated, and mineral spicules then grow parallel to this, within the protein fibres of bone (Glimcher, 1959), between them in calcified cartilage and in axiferan corals, and around them in the spicules of sponges, coelenterates, and echinoderms (yet with the crystal axis at an angle to the protein fibre (p. 167)). In typical external skeletons soluble protein and soluble calcium salts are secreted more or less simultaneously and precipitate together. The bird's egg shell compares with an external

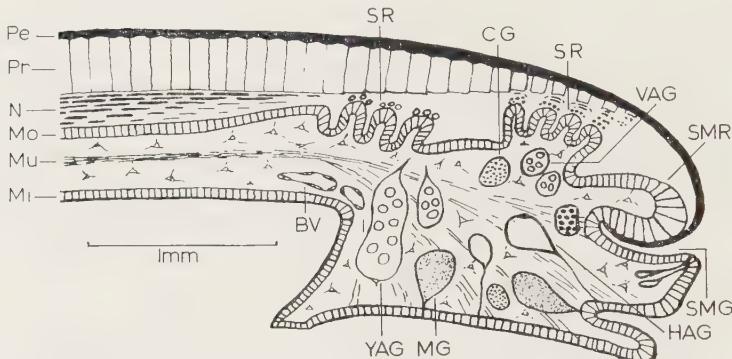


FIG. 13.4. DIAGRAM OF A SECTION OF THE EDGE OF THE SHELL AND MANTLE OF A GASTROPOD MOLLUSC, NORMAL TO THE SURFACE AND TRANSVERSE TO THE EDGE, TO SHOW THE MODE OF FORMATION OF THE THREE LAYERS OF THE SHELL

BV, blood vessel; CG, calcareous gland; HAG, homogeneous albumin gland; MG, mucus gland; Mi, inner epithelium of mantle; Mo, outer secretory epithelium of the mantle; Mu, muscle tissue in the mantle; N, nacreous layer of the shell; Pe, periostracum; Pr, prismatic layer; SMG, supramarginal groove; SR, secretion ridges with external secretion; VAG, vesicular albumin gland; YAG, yellow albumin gland.

(Based on Jones, 1935)

skeleton for the present purpose, whereas the calcification of the crustacean exoskeleton is somewhat anomalous (p. 176), and rather complicated.

In the formation of the molluscan shell, as a typical external skeleton, the outermost periostracal layer of quinone-hardened protein is secreted first, by cells at the bottom of a groove near the mantle edge. Since the periostracum remains anchored in this groove by its growing edge, a closed cavity is formed (Haas, 1929) between it and the mantle's outer surface (Fig. 13.4). Here, just behind the mantle edge, the mineral prisms of the second layer are formed, from the secretion of soluble protein and calcium salt (Jones, 1935). The same kind of mixture is secreted by corals (Picken, 1960) and other invertebrates. The protein forms nuclei on which the mineral deposits, and at an early stage the resulting calcospherites become attached, more or less evenly spaced, to the inner surface of the molluscan periostracum. Here again, therefore, the protein components play the essential part in initiation and orientation.

The fixed calcospherites grow in all directions until lateral pressure from neighbours converts them to pentagonal and hexagonal prisms, subsequently growing only normally to the surface. When the mantle edge region is carried forward by further growth of the mantle itself, the general mantle epithelium continues to secrete similar materials but in the now more confined

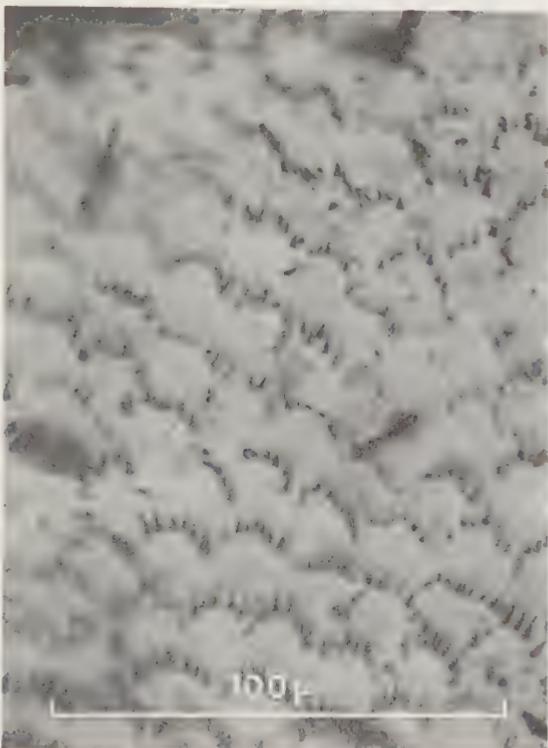


FIG. 13.5. SURFACE VIEW OF EXOSKELETON OF ISOPOD CRUSTACEAN, *Asellus aquaticus*, SHOWING CRESCENTIC ROWS OF MICROTRICHES, EACH CORRESPONDING TO ONE CELL OF THE EPIDERMIS BELOW

(Photo: P. L. Small)

space these are flattened into the typical flakes, composing the innermost, nacreous layer. It is one of the intriguing mysteries of the process that pigment, produced by other cells of the mantle edge (p. 65) becomes localized in the shell so precisely over the formative cells. This is particularly surprising because the prisms apparently do not correspond each to the field of one individual cell of the mantle epithelium. This could have been anticipated not only from the fact that deposition occurs freely in a fluid matrix but also because two distinct types of gland cell, at least, are involved, and that in any case these are large cells, sunken below the surface. There remains the possibility that the individual cells organize the actual deposition of the materials, and it

would be useful to compare the sizes of prisms and cells. Certainly there must be some device for keeping the materials localized during shell formation.

The egg shell of birds also is secreted in semi-solid form and precipitated *in situ*. In this case calcium and protein may be transported to the oviduct already associated as soluble calcium proteinate (Gutrowska and Mitchell, 1945). There is twice as much soluble calcium in the blood of laying as of non-laying hens.

For the crustacean exoskeleton the protein and mineral are passed out of the body in fluid form and precipitated *in situ* but the protein is orientated and

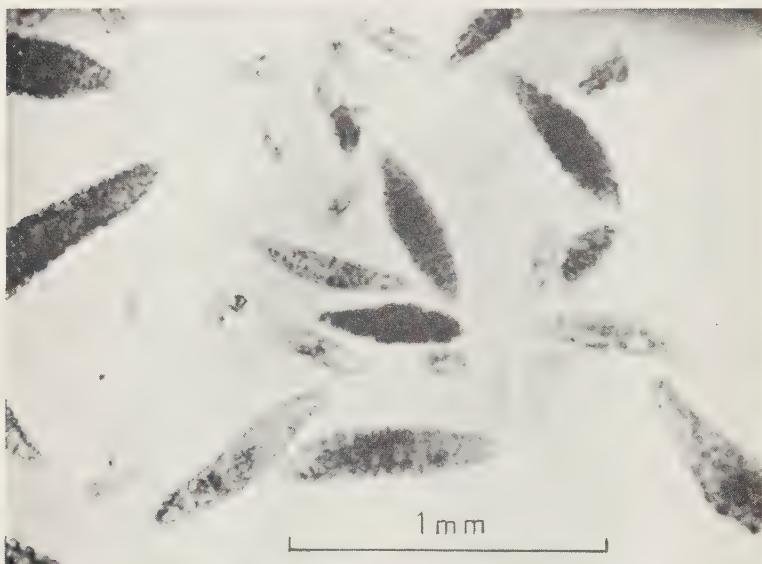


FIG. 13.6. SPICULES OF AN ALCYONARIAN TO SHOW THE TYPICAL TUBERCULATE SURFACE

(Photo: J. S. Haywood)

precipitated first. It is hardened by tanning in the epicuticle (Fig. 13.2), which therefore has some resemblance to the molluscan periostracum, and also in the outer layers of the exocuticle. It is stabilized by chitin in all layers except the epicuticle. It is deposited in polygonal areas (Dennell, 1960), which, unlike the molluscan prisms, do appear to correspond to the individual epidermal cells (Fig. 13.5); this vertical organization of the material is later obscured by the great development of horizontal layering, and by the mineral itself, which spreads out horizontally from foci of extrusion in the spaces between the polygons. Its crystallites tend to be radially orientated round these centres (Picken, 1960). The soluble calcium, like the protein, is secreted by the general epidermis. It eventually impregnates all but the deepest layers, precipitating in order from the most superficial inwards. The tanning agent, by contrast,

is secreted from special tegumental glands via ducts through the thickening exoskeleton, but its hardening progresses in the same direction as calcification. Setae and microtriches (Fig. 13.5) maintain cytoplasmic connexions through the exoskeleton.

It is possible to prepare models of a number of calcareous formations in the laboratory, using a mixture of an albumin solution, a soluble calcium salt and a soluble carbonate (Thompson, 1942). Spherical concretions or calcospherites form and grow until they make contact with neighbours, when the apposed faces flatten just as in the molluscan prisms. They also have concentric and

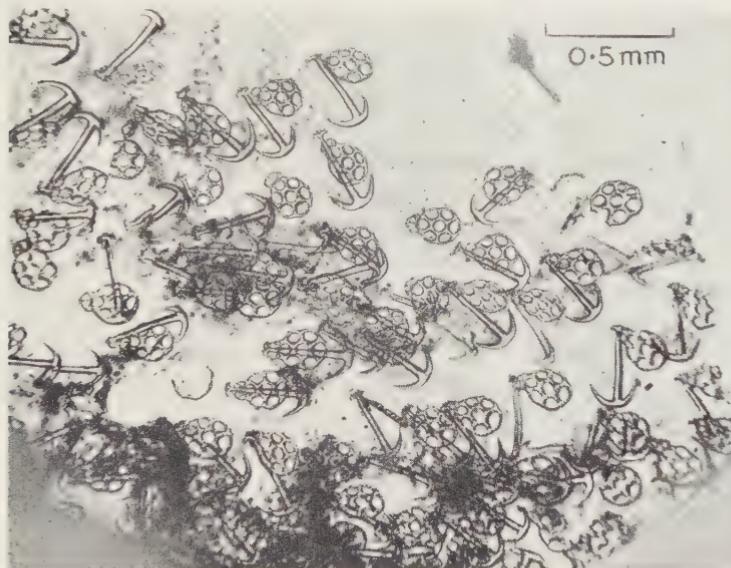


FIG. 13.7. ANCHOR AND PLATE OSSICLES FROM THE INTEGUMENT OF THE HOLOTHURIAN, *Synapta*, ILLUSTRATING THE GEOMETRIC SPECIFICITY OF FORM

Here each anchor is rather regularly associated with one plate.

(Photo: J. S. Haywood)

radial markings similar to those of some otoliths and of the calcareous granules of the tissues of trematodes and cestodes. Similar markings are seen in spherites which form by reaction between  $HgCl_2$  and soluble minerals from the exuvia of Crustacea (Needham, 1954). The radial markings are seen in the natural mineral depositions of the Crustacea. With lower concentrations of albumin irregular concretions are formed resembling the spicules of the Alcyonaria (Fig. 13.6), the Madreporearia and some Holothuria. The addition of a little calcium phosphate improves this resemblance. Some holothurians produce spicules of great geometric specificity, however (Fig. 13.7).

In bone and calcified cartilage the deposition of mineral in relation to protein already precipitated may have more specific demands than this. Apatite crystallizes only in collagen with the natural  $640\text{ \AA}$  periodicity (Edds, 1958;

Glimcher, 1959), and initially only in specific regions of the fibre. The mineral of Crustacea, however, seems less demanding (Richards, 1951; Dennell, 1960). This may be because it is mainly  $\text{CaCO}_3$  and not phosphate, but in fact there is some evidence that invertebrate mineral is laid down first as phosphate, and later exchanged for the carbonate anion (Polster, 1956). The high phosphate contamination in the completed crustacean exoskeleton would be consistent with this. There seems best evidence for this initial deposition as phosphate in the molluscan shell (Bevelander and Benzer, 1948; Polster, l.c.). This is a typical case of external co-precipitation, and so would seem to decrease the value of the albumin-carbonate model. The effect of phosphate on this model therefore deserves further study. If initial phosphate deposition is the rule then the vertebrate materials are unusual only in retaining and not replacing the phosphate, and they may be considered along with the rest.

Carbonate is not very readily precipitated at low temperatures because of the high solubility of respiratory carbonic acid. It is also more soluble in the presence of soluble proteins than in simple non-biological systems (Thompson, 1942, p. 669) whereas the solubility of phosphate is not greatly changed. More positive evidence for initial  $\text{PO}_4^-$ -deposition is that snails store calcium phosphate and not carbonate in their digestive gland and transport it to the mantle when shell is being formed. Moreover, alkaline phosphatase, which liberates inorganic phosphate,  $\text{P}_i$ , from organic compounds, including ATP itself (Danielli, 1951), is very active in the relevant tissues, not only of vertebrates but also of molluscs (Bevelander and Benzer, l.c.; Fretter, 1952; Polster, l.c.), serpulids (Hanson, 1948), and Madreporaria (Goreau, 1956). Its concentration is also high in the serum of laying hens (it is to be noted that egg-shell formation is very much a systemic activity (p. 180)).

One advantage of phosphate is that it can be provided from an organic phosphoryl compound, making energy available for the process at the same time. Glucose-1-P and glycerophosphate have been shown to promote calcification in vertebrates (Hevesy, 1948, p. 410) and any phosphorylative breakdown of glycogen leads to accumulation of  $\text{P}_i$  in the epiphyseal cartilage (Glimcher, 1959). In bone at least the evidence for an organic P precursor is good, although *in vitro* salts such as  $\text{CaHPO}_4$  will deposit apatite, in alkaline medium. The concentrations of  $\text{Ca}^{++}$  and  $\text{HPO}_4^{=}$  ions in vertebrate body fluids are, in fact, permanently above their solubility product, and there appears to be some mechanism actually preventing their direct co-precipitation. Consequently sodium phosphate is incorporated into bone as readily as the soluble Ca salt. In molluscs, therefore, inorganic phosphate may be stored not as the direct precursor of the initial skeletal material but simply as a suitable form for subsequent mobilization, probably as a soluble organic phosphate.

There are a number of reasons for thinking that ATP is in fact the immediate source of P and of energy, those carbohydrate P-esters which promote mineralization therefore serving indirectly as intermediaries for the rephosphorylation of adenosine diphosphate (ADP)—as they do in muscle metabolism and most

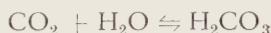
metabolic processes. Calcium is an activator of ATP-kinase, the enzyme which mobilizes the terminal P from ATP, so that there might result a co-precipitation of Ca and PO<sub>4</sub>. The relatively large amounts of magnesium, another activator of ATP-kinase, in skeletal materials is consistent with this idea. Again, beryllium and copper ions are powerful inhibitors of calcification (Glass, 1952) and are found to inhibit a number of P-mobilizing enzymes (Dixon and Perkins, 1956).

In view of the positive action of alkaline phosphatase on the formation of fibrous proteins as well as on mineral deposition, it is interesting that beryllium appears to inhibit regeneration mainly through an action on the initial process of proteolysis (Needham, 1960a). During wound healing in mammals there are two periods of maximal alkaline phosphatase activity, one in the early demolition phase and one later, during the phase of synthesis of fibrous proteins (Bourne, 1956).

Ascorbic acid (p. 294), which is very frequently associated with alkaline phosphatase activity, presumably as a neutral or alkaline salt, also promotes bone growth. Glutathione, GSH, which forms a natural reducing system with ascorbic acid, also is a promotor. Both agents are highly concentrated in the adrenal cortex and it is significant that the adrenal cortical hormones antagonize their action on bone growth, through an effect on oxidation-reduction processes. The hormones are also known (p. 369) to promote general proteolysis, so that again a duality or multiplicity may be anticipated in their total action.

In the vertebrates, at least, the levels of soluble Ca and P in the body are so nicely balanced that P must be deposited or excreted when Ca is precipitated, but it is not certain if this would also explain an initial deposition of P in invertebrates. The shell of molluscs is very sensitive to CO<sub>2</sub> concentration and is dissolved under anoxic conditions; it is likely, therefore, that an initial deposition of CO<sub>3</sub> might be an uncertain process under the relatively reducing conditions associated with an active ascorbic-GSH system. Deposition of calcospherites by molluscan tissues is possible only within a very narrow range of CO<sub>2</sub> concentrations (Wagge, 1954). In vertebrates Ca is not deposited as CO<sub>3</sub> even if there is a deficiency of PO<sub>4</sub>, but as citrate.

If the initial mineral were carbonate a high concentration of carbonic anhydrase might be expected at the site. In fact, there is little in molluscs (Freeman and Wilbur, 1948) and its level of activity does not change during the moult cycle of Crustacea. The enzyme catalyses the reaction—



so that it might have been expected to promote the precipitation of insoluble carbonate under alkaline conditions. The position concerning the enzyme is not altogether clear, since it does seem to be active in the calcification of corals (Goreau, 1959), in mollusc shells which are growing rapidly (Freeman, 1960) and in barnacles (Costlow, 1959). In laying hens inhibitors of the enzyme result in soft-shelled eggs (Gutrowska and Mitchell, 1945); use of the enzyme here might explain how it is possible to form a carbonaceous egg shell in a

body which normally produces phosphatic skeletal materials, but it raises the problem of the significance of the increased serum phosphatase activity in the laying hen, and of the observation (Hevesy, 1948) that  $\text{PO}_4$  exchanges between blood and egg shell as long as the egg remains in the oviduct. No doubt there is a very profound interrelationship between the  $\text{CO}_3$  and  $\text{PO}_4$  metabolic systems. In Crustacea the ratio of  $\text{PO}_4$  to  $\text{CO}_3$  determines the mineral form of the latter: above a ratio of 1/10 no crystalline but only amorphous carbonate is deposited (Richards, 1951). This may be why the carbonate in bone is amorphous (p. 164).

Phosphate skeletons are harder, stronger, and less soluble than carbonate and it is therefore surprising that they have not been more extensively exploited as the definitive material. Possibly phosphate is too precious in most invertebrates to be squandered in the skeleton. It may be significant that bone salts are readily available for use elsewhere in the vertebrate body when necessary while the egg shells, which are lost to the body, contain only  $\text{CO}_3$ . On the other hand laying hens excrete over 50 per cent of the P they ingest (Hurwitz and Griminger, 1961) so that there appears to be no real shortage. The whole subject bristles with piquant problems.

Mucopolysaccharides appear to inhibit the deposition of apatite (Glimcher, 1959) and this may be one of their main functions in cartilage. When hyaline cartilage calcifies they are first depolymerized, as shown by changes in metachromatic staining; their concentration in bone is low. However, it is thought that they may promote calcification in serpulid tubes (Hedley, 1956, 1958). Chondroitin sulphuric acid readily chelates Ca and this plays a part in the formation of the egg shell of birds (p. 176). Conceivably this polysaccharide may assist or prevent deposition according to conditions, for instance according to the nature of the final anion.

It was once supposed that since 18 ions must contribute to one molecule of apatite (p. 163) there must be a long series of intermediary chemical reactions, but the process of formation is probably more akin to crystallization (Glimcher, l.c.), the ions being added individually and separately. The final product approximates to apatite statistically rather than in precise molecular structure, and other ions readily replace some of the major ones at any stage, and to varying extent. This may simplify the problem of replacing the initial  $\text{PO}_4$  by  $\text{CO}_3$  in invertebrate skeletons. The deposition of these minerals has something of the lability and heterogeneity of the growth of the organic components, perhaps owing mainly to the influence of the latter. For instance the  $\omega\text{-NH}_2$  group of lysine in the protein appears to be involved in the calcification of bone (Glimcher, 1959).

Owing to the large amounts of skeletal material synthesized it is relatively easy to study systemic aspects of this type of process. It is evident enough that for intracellular growth, also, there must be systemic distribution of the raw materials and the controlling agents, and knowledge of this has increased greatly since the advent of tracer techniques (p. 186) but the picture is less complete than

for skeletal and other special materials. Where the skeletal deposition is sharply periodic, as in the moult cycle of Crustacea (Knowles and Carlisle, 1956) and in the egg-laying of birds, there are dramatic systemic changes. The bird stores calcium in the bone marrow for use in shell formation, though it is also capable of increasing its retention of dietary Ca, so that as much as 60 to 70 per cent may come from that immediate source. Its serum calcium level rises and also that of alkaline phosphatase, as already noted.

Virtually every aspect of crustacean metabolism seems to be involved in the moulting and reformation of the skeleton. Glycogen is mobilized from the digestive gland and transported as glucose for conversion to chitin peripherally. During intermoult some of the larger Crustacea store calcium as the gastroliths of the stomach lining. The level of serum calcium rises during the moult, and mineral is also partially salvaged from the old exoskeleton. Apart from the oenocytes of insects and the tegumental glands of Crustacea few types of cell other than the general epidermis have been shown to play a part in the final process of deposition of exoskeleton, notwithstanding its large number of constituents; this is, therefore, a further indication that many are synthesized systemically and simply transferred by the epidermis. This accumulates droplets of material at the onset of moulting (Needham, 1946).

There is a parallel to this in the mammary gland (Folley, 1952) where again one type of cell either produces or transports a wide variety of materials for secretion. Some of these, the lipids in particular, are definitely products of the cell itself and are continuously secreted by apocrin of the distal part of the cell. Lactose is peculiar to milk and so also may be synthesized in the gland. Other materials are equally certainly synthesized elsewhere in the body. The total output of the gland cell is outstandingly high, about 15 times its own mass per day; even such an active cell as the pancreas acinar cell secretes only its own weight per day (p. 117). Owing to this high rate the mammary gland has proved as useful for the study of productive synthesis as skeletal structures, and is very relevant here.

In molluscs, amoebocytes play an important part in the systemic mobilization of mineral from stores in the digestive gland and elsewhere (Wagge, 1954). During regeneration of the shell they actually deposit the material *in situ*, an interesting difference from the initial mode of formation (p. 174). *In vitro*, shell formation by mantle tissue is scanty, perhaps owing to the absence of this systemic mechanism, though the dedifferentiation of the mantle cells is an alternative possibility.

Less is known about silica deposition than about  $\text{PO}_4$  and  $\text{CO}_3$ . The sarcodine protozoon, *Difflugia*, forms siliceous plates in its cytoplasm and then marshalls them to the periphery to form the external shell. Siliceous sponge spicules are built on an organic primordium but there is little or no organic material interleaving the successive layers of mineral (Hyman, 1940) as there is in calcareous spicules. Siliceous spicules originate intracellularly, stored granules being dissolved for the purpose (Jørgensen, 1947). They outgrow the cell, as

do the calcareous spicules of sponges, coelenterates, and echinoderms, and many adventitious cells contribute to further growth. The hexactinellid, *Monorhaphis*, builds a rooting spicule 2 to 3 metres long and 1·2 cm in diameter! Spicules may become compacted by a cement of silica or of the organic material, spongin; in the Keratosa spongin has completely replaced silica. It likewise originates intracellularly, outgrows the cell, and is augmented by the activity of adventitious cells. The radiolarian skeleton (Fig. 13.1) is deposited both extra- and intracellularly, and even in the nucleus.

Silica has a low solubility, but sponges can accumulate it from a solution as dilute as 0·4 per cent (Jørgensen, 1947). It is deposited easily, even at low temperatures, so that siliceous sponges thrive in high latitudes where the Calcarea would be unable to precipitate  $\text{CaCO}_3$ .

Skeletal materials, once formed, are not dead but undergo a slow process of metabolic turnover which may be related to repair, remodelling and other aspects of growth, as shown in the next chapter. The turnover is most rapid in the young and therefore is due largely to growth processes. It is less rapid than in metabolically active intracellular materials. Taking the turnover rate in the collagen of adult rat tendon as unity, Neuberger and Slack found the rates in the collagens of skin, liver, and bone to be 1·4, 2·6, and 4·6 respectively (Picken, 1960, p. 380). Those for the same four tissues in young rats were 32, 83, 189, and 169 respectively, and illustrate the usual age change, but even these values are small compared with figures up to 2,800 for other liver proteins. The low values probably reflect the high stability of collagen and its resistance to wear of all kinds. Turnover is even slower in the mineral than in the organic component of skeletons, perhaps because the mineral is even more stable. The danger of radioactive  $^{90}\text{Sr}$ , acquired from fall-out, is that as a calcium analogue it is rapidly deposited in the young but much more slowly replaced (by normal Ca) in later years. The superficial layer of flesh, or coenosarc, of corals appears to protect the mineral of their skeletons from exchange processes (Goreau and Goreau, 1961), an interesting example of the way morphological features may control activity at the molecular level.

## CHAPTER 14

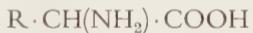
### *The Biosynthesis of Proteins*

“The very molecules appear inspired with a desire for union and growth.”

JOHN TYNDALL

THE order of treatment adopted in Part I is the historical order (p. 7), mainly because investigation becomes progressively more difficult with decreasing order of magnitude. Although the youngest field, however, research at the molecular level has the benefit of new and penetrating techniques and of the increasing tempo of research. In particular, the technique of labelling raw materials with the heavy or the radioactive isotopes of key atoms has already yielded rich dividends. Attention here will be focused mainly on the biosynthesis of molecules of protein and nucleic acid, since these are the most abundant and the most important constituents of the living fabric. Moreover their syntheses illustrate most of the general principles. In the synthesis of other molecules, only those general features necessary for an understanding of other sections of the book will be considered.

There is little doubt that all proteins are polypeptides, that is to say mixed or heteropolymers of amino acids (a.a.s). Further they are polymers mainly of a particular set of twenty  $\alpha$ -amino acids, having the generic formula—



All are L-amino acids, structurally related to L-glycerose. The smaller quantities of other a.a.s sometimes present, and often peculiar to particular taxonomic groups, may be ignored in the first instance. Polymerization of a.a.s is by a condensation reaction in which the  $\alpha$ -NH<sub>2</sub> group of one loses an H atom and the COOH of the next an OH group; it is not a simple salt formation, therefore. In fact the NH<sub>2</sub> group behaves as an acid and the COOH as a base. The bond is known as a peptide bond and proteins are built of polypeptides, linearly, or rather spirally, repeated condensations of this kind. Some peptide chains close to form a ring. The polypeptide chains are the starting point for the further polymerizations considered in the previous chapter.

Autotrophes can build all their a.a.s by reducing and combining simple, fully oxidized, inorganic materials such as CO<sub>2</sub>, H<sub>2</sub>O, nitrates, sulphates, and phosphates, but heterotrophes synthesize only about half of the twenty, the so-called non-essential group, and these mainly from near precursors, which

include the remaining, essential members (p. 285). These are demanded ready-made in the diet, though some are in part synthesized in the body. For heterotrophes, therefore, protein synthesis virtually begins with a metabolic pool of dietary amino acids and the synthesis of the latter may be taken for granted; a recent account is given by Kit (1960).

#### 14.1. The Intake of Amino Acids into the Body

This may be regarded as a tentative model for the intake of raw materials in general; it is also an important step in the synthesis (Davis, 1956). All the common  $\alpha$ -amino acids are readily taken up from the gut of animals into the blood stream; the tissue cells in turn quickly absorb them from the blood, and from suitable media *in vitro* (Borsook, 1953). Unusual a.a.s, and the D-isomers of the common ones, are taken up much less rapidly; the intake is selective, therefore, and this is true quantitatively also, so controlling the whole pattern and tempo of synthesis and of metabolism in general (Davis, 1956). Intake by Gram-negative bacteria depends also on the subsequent fate of the a.a.s (Gale, 1953, 1959a) so that there is also a reciprocal control, of intake by synthesis. Very often there is intake only so long as there is a net synthesis of protein (Davis, 1956). Incorporation into large molecules could promote further intake by simple mass-action effect. Gram-positive bacteria, however, appear to build up a pool of a.a.s in the body as the mammals do, so that further intake is less directly dependent on their immediate use in protein synthesis. In some cases it is uncertain whether intake or subsequent utilization is the limiting step (Davis, 1956).

If a single species of a.a. is presented to Gram-positive bacteria, it may be absorbed and concentrated by a factor as great as 400 times (Gale, 1953) but in this case to be used as fuel or for building the cell wall (Gale, 1959a). Presentation of two or more acids causes a much lower intake of all, largely because the a.a.s mutually affect the intake of each other and also because they interact to form oligopeptides which accumulate in the external medium (cf. p. 111). With further increase in variety the total intake again increases; complete proteins are now synthesized and retained. This illustrates the importance, for sustained synthesis, of a simultaneous presentation of all the a.a.s (Geiger *et al.*, 1949), notwithstanding the resources of the pool. Young growing animals are most sensitive to this requirement. Unbalanced mixtures of a.a.s are more extensively catabolized, if only because complete proteins are more stable than peptides; this stability is a very important property in promoting synthesis (pp. 194, 231). A further factor of importance in the control of intake is the specific interaction between particular a.a.s (Gale, 1953; Christensen *et al.*, 1952; Borsook, 1953; Horowitz, 1956). Such pairs as leucine and valine, and aspartic and glutamic acids, being members of the same homologous series, compete for active sites of intake (Gale, l.c.), though the intake of glutamic acid, in fact, is also depressed by alanine, glycine, and cysteine. Leucine completely inhibits the intake of its homologue valine, so that the effect is not

merely in proportion to their relative concentrations; in this case something more than mere competition between the two is involved (Monod, 1956). The formation of peptides outside the cell itself may be a common mode of interaction, these peptides then permeating less readily than the free a.a.s.

Not all interactions inhibit intake, however, (Christensen *et al.*, *l.c.*). Valine, leucine, and isoleucine promote the intake of glutamic acid and the diamino acids promote that of most other amino acids. For this reason it is probably significant that pyridoxal, co-transaminase (p. 311), is an active promotor of intake (Riggs *et al.*, 1953). Histidine and phenylalanine also improve the uptake of other amino acids in general, and it is interesting that lysine, histidine, and phenylalanine all play special parts in synthesis, at the macromolecular level (Chapter 13). It may be presumed, tentatively, that the complete complement of all the common a.a.s constitutes a system of controlled intake, with promotor and depressor actions nicely balanced.

The intake mechanism is not identical for the different a.a.s. That of lysine, glycine, alanine, and arginine into Gram-positive bacteria (Gale, 1953, 1954) is relatively passive, but that of glutamic acid, aspartic acid, proline, methionine, valine, and phenylalanine involves metabolic activity, so that it is inhibited by respiratory poisons and by penicillin, and is very sensitive to temperature changes. It is part of the cost of growth (p. 252), therefore. This type of intake reaches a ceiling value as the concentration of the a.a. presented is increased, whereas passive intake has no limiting rate. The latter is associated with a change in the electrical charge on the cell surface (p. 409) and appears to be a more physical process, therefore. Borsook (1953) recognized two distinct mechanisms of intake even for the same a.a., lysine, into the mammalian liver cell: that into the supernatant cytoplasm is passive but that into the mitochondria is sensitive to oxygen tension. This difference may be related to a difference in subsequent fate of the acid in the two locations. The active type of intake is promoted by ATP, and by the  $Mn^{++}$  and  $Mg^{++}$  ions, which activate ATP-kinase. Arsenite, a competitive analogue of phosphate, completely inhibits the intake into some mammalian cells, though not into *Escherichia coli* (Borsook, 1953). DNA is necessary for intake by the nucleus (Allfrey *et al.*, 1960) and this once more emphasizes the close link with subsequent synthesis. RNA may be expected to control entry into the cell.

Active intake implies enzyme action and there is, in fact, evidence for special "permeases." They resemble inducible enzymes (p. 208), depending for survival on the continued presence of their amino acid substrate and becoming diluted out over the generations when this is withheld (Davis, 1956; Monod, 1956). Unlike other enzymes, permeases probably cause no permanent change in their substrate. There may be some relationship between permeases and transferases (p. 303) since, in addition to pyridoxal, other B-vitamin coenzymes promote a.a. intake, for instance nicotinamide, riboflavin, and pantothenic acid. There may even be a close relationship to protein synthesis, the a.a.s being activated in the same way (Meister, 1959).

A.a. intake necessitates the movement of the  $\text{Na}^+$  and  $\text{K}^+$  ions across the membrane, in opposite directions. The  $\text{K}^+$  ion is the more critical and the movement of  $\text{Na}^+$  may be regarded as compensatory. Some a.a.s cause  $\text{K}^+$  to move in and others out (Gale, 1953; Riggs *et al.*, 1953). A balanced presentation of all a.a.s, therefore, may cause only a small net movement, which is an inward flux of  $\text{K}^+$  (p. 291). Protein synthesis in the cell also involves the transfer of these ions across the membrane (p. 271), and this is far from peculiar to nerve conduction, therefore. It has been suggested (Hodgkin, 1951) that a.a.s act as carriers for these ions in nerve conduction, and reciprocally the neural tropic effect on growth in end-organs (p. 352) may depend on ion-induced a.a. movement. The relation between the two may be fairly complex since the rate of intake of some inorganic ions is a linear function of their concentration (Steward and Millar, 1954) while that of some of the a.a.s is proportional to the logarithm of their concentration (Borsook, 1950). The transport of a.a.s across the nuclear membrane requires  $\text{Na}^+$  and not  $\text{K}^+$  (Allfrey *et al.*, 1960). In explanation of this it may be pointed out that  $\text{Na}^+$  is relatively concentrated in the body fluids of animals and  $\text{K}^+$  in the cytoplasm. At the same time it must be admitted that  $\text{K}^+$  is necessary also for the entry into bacteria from the external medium.

Infants absorb droplets of their mother's colostrum, and such absorption of large peptide molecules from the gut is probably not rare (Fisher, 1954). Antibodies acquired in the colostrum are effective only if their molecules remain intact; foreign proteins are usually broken down far enough to make them non-antigenic, if not as far as free a.a.s. Tissues, for instance the mammary gland, take up whole protein molecules from the blood stream (Madden and Whipple, 1940; Potvin, 1951), and proteins pass both ways across the placental barrier (Bangham *et al.*, 1960). The bar to absorbing intact food proteins more extensively, therefore, appears to be biological rather than physical: they are structurally unsuitable for direct use and usually they are positively dangerous, as antigens.

The uptake of a.a.s and other materials is a well-controlled process, specific to biologically useful materials, and differential even among these. In some respects the control is automatic. A.a.s interact with each other and their intake is often bound up with their subsequent fate; it is very much part of biosynthesis. It has also much to teach about the properties of membranes.

#### **14.2. The Incorporation of Amino acids into Proteins**

The use of labelled amino acids (Schoenheimer, 1942; Hevesy, 1948; Sacks, 1953) has already revealed a great deal about their normal fate in the body, and the major problem is to find out how far this is connected with growth and related processes. The uncertainty whether the labelled atom stays with its initial molecule has been dispelled. Hydrogen attached peripherally, as it were, by a labile bond to an O or a N atom may move to another molecule, but H bound to the C atoms of the skeleton of organic molecules

"stays put," and this is true of elements in general bound to the C skeleton, including C itself of course. Such atoms may be used as reliable markers, therefore. Some caution is necessary, since the two marked atoms of a molecule which has been doubly labelled, for instance leucine and lecithin, do seem to show partial independence in their subsequent movements. However, the lecithin molecule, at least, may be split in the course of metabolic activity, and with precautions the double label may be used to elucidate this. With the further precaution of measuring total intakes and outputs, and with other cross-checks, the technique is as reliable as it is elegant and convenient.

Sulphur, phosphorus, iodine, iron, and magnesium appear to exchange rather readily as individual atoms, or radicals, no doubt because they are often held by labile, reactive bonds. The Goreaus (p. 182) have shown that Ca of the skeleton is equally labile, but also that, in a simple mechanical way, a layer of flesh may depress its exchange rate with Ca of the medium. At the same time there is some evidence that these exchanges are biochemically significant, and not simply physical, since they occur only from or to biological materials and not from inorganic compounds. The general conclusion seems to be that a label on a reactive atom or radical will trace the metabolic movements of that group while one on the nucleus or backbone of an organic molecule will trace this as an entity.

When a labelled a.a. is taken up by the body, and retained, it is found to have been incorporated into the proteins. This is relevant to growth, therefore, but it proves to be much too extensive to be concerned only with the positive increase in mass of body proteins, and is extensive even in adults. Classical studies on adult metabolism had indicated that only a small percentage of the a.a.s of the food passed into the fabric, enough to replace a small and very steady amount of loss and wear, measured by the rather constant rate of excretion of creatinine and of neutral sulphur. This is the so-called "endogenous" metabolism, 3 to 4 per cent of the total nitrogen metabolism, and the "exogenous" metabolism experienced by the vast bulk of the a.a.s of the food was believed to be simply their breakdown as a source of energy. Tracer work, however, indicates that as much as 50 per cent of ingested labelled a.a.s may pass into the body proteins (cf. Chapter 16), therefore displacing an equivalent amount of unlabelled material for eventual excretion: the term "metabolic turnover" has been applied to this rapid flux. If the dietary intake is increased, metabolic turnover increases in parallel (Solomon and Tarver, 1952), and gives the impression of an automatic flow-through, which is simply tapped off as required for growth and repair. For a time after a period of starvation all food nitrogen is retained (Carter and Thompson, 1953, p. 205), presumably because a large debt has been incurred in the tissues and body fluids, and all is now tapped for its repayment, as repair. The problem of measuring the components associated with "normal" growth and repair, therefore, presents some difficulties. True growth, the synthesis of new molecules, is called *neoformation* and any turnover not due to this is thought to involve the exchange of the dietary a.a. for its

identity, damaged or intact, in the fabric proteins. Repair at any level above that of the individual a.a.s would, of course, necessitate protein neoformation.

It has been found that in actively growing animals, and organs, true growth constitutes at least a major fraction of turnover. The turnover of DNA in four-day-old rats is twenty times that of adults (Hevesy, 1948, p. 349) and the  $^{32}\text{P}$  uptake by growing yeast is twenty times that of the resting cells (Haven and Hodge, 1941). In tumour tissues the uptake of glycine and alanine into nucleoproteins is six times normal (Zamecnik *et al.*, 1948). Turnover in the epiphyses of the long bones, including the formative zone of the shaft (p. 54), is three times as high as in the diaphysis, and there is a similar difference in rate between the basal and distal regions of growing teeth. Turnover is high in the pancreas and other productive tissues. There is a progressively lower rate in the series, intestinal mucosa, kidney, liver, plasma, pancreas, lymph nodes, spleen, heart, muscle, and brain (Sacks, 1953), which shows a general parallel to the combined growth and productive activities of the tissues. The low rate in the collagen of the connective tissues has been noticed previously (p. 182).

That a large fraction is used for true growth is indicated also by the finding (Borsook, 1953) that known inhibitors and promoters of growth, and of protein synthesis affect a.a. uptake and turnover very similarly. Turnover is promoted by a number of hormones, by nucleic acids, by a number of the B-vitamins and by aerobic respiration. Heavy metals inhibit a.a. incorporation as they do peptide synthesis (p. 193).

Further analysis, however, has clearly demonstrated a component of turnover not concerned with neoformation, and in some cases a quantitative measure of each has been obtained. Thus aureomycin and other antibiotics inhibit completely, and arsenite substantially, the growth of certain micro-organisms, in concentrations which have no effect on the incorporation of glutamic acid into the cell proteins. Similarly ethionine, a competitive analogue of the a.a. methionine, inhibits protein synthesis at a much lower concentration than that required to arrest the uptake of methionine specifically (Francis and Winnick, 1953). A possible explanation is that exchange is not so easily inhibited as neoformation. If the mechanisms for the two are different then, reciprocally, it should be possible to find agents which inhibit exchange more than neoformation, and certainly arsenite appears to inhibit a.a. uptake in general, by mammalian cells, more than it inhibits growth (Borsook, 1953). Exchange demands the simultaneous rupture, and reformation, of two peptide bonds, whereas neoformation may involve the formation of only one at each step, so that there are good reasons why exchange might be the *more* sensitive to inhibition (Borsook, 1950). Of course it is necessary to know that a.a. uptake does measure the actual incorporation into protein and not merely the preliminary intake into the body's pool of free a.a.s; protein synthesis from this pool might continue for some time after further intake into the pool had been inhibited.

There is further evidence for the reality of molecular, and even intramolecular, exchange from the fact that the rate of turnover of the relevant units is differential: it is clear that in neoformation they must be incorporated at the same specific rate. Glycine and phosphorus turn over at different rates in DNA (Le Page and Heidelberger, 1951), and choline, fatty acid, and phosphorus at different rates in lecithin (Hevesy, 1948, p. 247). The differential rate found for glycine, between haemoglobin and the stroma proteins of erythrocytes, is not unequivocal evidence since the two proteins may have a differential rate of neoformation and also a different proportion of glycine. There are many equivocal examples: as critical evidence it is necessary to demonstrate, as in the first examples above, a differential rate between the constituents of the same macromolecule, differing also from their normal proportions in the molecule. Few of the recorded examples are completely satisfactory on this score.

There is some evidence that a.a.s presented singly may participate only, or at least mainly, in exchange (Gale, 1953; Gale and Folkes, 1954), whereas a good spectrum of different a.a.s offered together cooperate extensively in neoformation (Haurowitz, 1952). Exchange appears to be slower than neoformation (Friedberg *et al.*, 1947; Deasy *et al.*, 1949), which is consistent with its two-bond requirement.

Neoformation and exchange are not entirely unrelated. RNA is thought to be essential for the transfer of a.a.s in both (Hoagland, 1959). The anterior pituitary hormone, APGH (p. 365), and other agents which promote growth do so not by increasing neoformation, specifically, but by depressing the outflow component of turnover, normally used as the measure of exchange alone. Presumably a.a.s which previously were merely displacing their identity in the fabric are now taking part in neoformation. Much the same conclusions may be drawn about the response following a period of starvation (p. 187). In the laying season female birds retain a larger percentage of their dietary Ca, to be used for egg-shell formation (p. 179), showing that the phenomenon applies also to inorganic elements.

Some workers in the field (Hogness *et al.*, 1955; Cohn, 1957) believe that turnover is concerned only with growth and that there is no mere exchange or passive flow through. Bacterial proteins are often very stable, once formed, and do not show any subsequent turnover. Stable materials such as collagen also show little subsequent turnover. That of DNA is very low as soon as nuclear proliferation ceases (Pelc and Howard, 1952; Allfrey *et al.*, 1955a), however active the tissue may be physiologically. There is no turnover in the molecules of antibodies, once they are completed (Taliaferro, 1957). Mammalian erythrocytes, as an example of cells which are physiologically active but not growing, and non-productive, show a very slow turnover when mature.

Productive cells maintain a high rate, and it is generally agreed that any type of synthesis does involve active turnover (Cohen, 1959). It is also generally conceded that the replacement of damaged cells may cause a positive rate of

turnover, even in tissues with no net increase in mass, for instance the skin and haemopoietic tissues. If the concession were to be extended to the replacement of damage at the molecular level then the difference between this and the orthodox view would narrow down to the question whether there is a component of exchange which is not relevant even to molecular repair.

It is probable that there is a good deal of repair at the molecular level. Enzyme molecules may have a life as short as five minutes (Haldane, 1954, p. 111), and this tallies with the estimate by Morel (1941). The molecules of nicotinic acid may survive in their coenzyme form (p. 225) for as long as eight days but this is still in the general range of observed turnover rates. A higher turnover has been found in recently active than in resting muscle (Hevesy, 1948), no doubt measuring the increased damage due to use. Antibodies begin to show turnover once more when they are released into the body for action against their antigens (Taliaferro, 1957). Turnover is very active in systems which are degrowing or experiencing any other type of accelerated breakdown, for instance in *Amoeba* (Mazia, 1956) and other animals when starving. Their rate of uptake of materials also increases, if facilities are available, even though it may be unable to keep pace with losses. In bacteria turnover begins to increase as the population size becomes stationary, and the death rate rises to equal the proliferation rate (Hinshelwood, 1957). There is a rapid turnover even in detached leaves (Chibnall and Wiltshire, 1954). In some of these examples the turnover includes some wear at the cell level, but there is little doubt that the fraction due to damage and repair at the molecular level is considerable. Simpson (1953), Steinberg *et al.* (1956), Walter (1960) and others have shown that there is a type of breakdown for reuse which is quite different from the familiar proteolysis of digestion.

If we include this item as repair, then growth and repair together might be considered adequate to account for the whole of the recorded metabolic turnover in the fabric of the body. Nevertheless, it does seem that there may also be a gratuitous or floating component, not due to damage and wear on the outflow side or to neoformation and repair on the inflow side, though available, as required, for growth and repair. It is a variable component (p. 187), even before it is tapped in this way for growth (p. 188), so that its outflow would formerly have been included in the exogenous component of metabolism, the essential criterion for which was a variability in amount from day to day. Damage and wear also vary and so were likewise credited to exogenous metabolism: hence the low values given for endogenous metabolism. It would be logical to equate metabolic turnover *in toto* with endogenous metabolism, which therefore is both a major and a variable component of metabolism. Independent evidence that it is a major component is the observation that after the first few days of a fast the nitrogen output of a mammal settles down to a steady value not far below that for the normal, feeding animal. The fasting animal is known to be obtaining more than 80 per cent of its energy from fat reserves, at this stage, so that the high N-output is probably due

mainly to the normal processes of wear, and not as yet to an extensive use of body protein as fuel. It will be noted that under these conditions the endogenous component is not only large but also constant, food being a major cause of variation in the normal animal. It was well known that increased food intake led to increased use of amino acids as fuel, which is true exogenous metabolism, and therefore it is not surprising if all the variation was attributed to the exogenous component. The amount of uric acid excreted fluctuates with the protein intake and this at any rate appears classically to be regarded as a part of endogenous metabolism (Hawk *et al.*, 1954, p. 1052). However, the nucleic acids of the food contribute to excretory uric acid and in birds so does the excess amino nitrogen.

Although the exogenous component may involve intracellular metabolism, it does not concern the growth and maintenance of the cell's fabric directly. Consequently "fabric metabolism" might be an alternative synonym for endogenous metabolism or metabolic turnover. Its balance sheet might be represented—

$$\text{Inflow} = \text{neoformation} + \text{repair} + \text{variable exchange fraction}$$

$$\text{Outflow} = \text{damage} + \text{wear} + \text{variable exchange fraction}$$

The rate of incorporation of a.a.s into living systems is slow by laboratory standards (Borsook, 1950), but test-tube reactions are not "natural" since manufactured and purified reagents are used, too reactive to be found free in nature. In fact, turnover *in vitro* can be very rapid: the exchange of some molecular species is complete in twenty seconds (Hevesy, 1948, p. 269). In the mammalian body the normal rate is estimated as between 0·1 and 10·0  $\mu$  moles of a.a.s per gramme of body protein per hour, that is between 10 and 1,000  $\mu\text{g}$ , taking 100 as the average molar weight of an a.a. If the protein content of an average adult man is 11 kg the turnover would lie between 2·64 and 264 g per day, which more than covers the actual range of amounts of food protein assimilated by human races. Haurowitz (1952) gives the range 15 to 20 grammes of protein as the amount incorporated daily and the figures of Rittenberg (1951) lead to the value 28 g for an average, 70-kg, man; this is around the geometric mean of Borsook's range. Generous diet sheets allow about 100 g of protein per day, of which 20 to 40 g may be assimilated (p. 254), so that this is a reasonable allowance. However, as already noted (p. 187), to some extent turnover reflects intake rather than requirement. On these figures complete renewal of the body proteins by exchange would occupy between 40 and 4,000 days (11 years), ignoring the possibility of repeated exchange of some molecules, and this covers the range of estimates made prior to the tracer era. Like metabolic rate, heart beat (p. 27) etc., turnover is faster in small than in large mammals (Taliaferro, 1957).

The rate as measured by the half-life of labelled materials, the time which elapses before a single dose of the label has been half eliminated again, usually lies near the upper end of the range. Labelled glutathione has a half-life of only

four hours (Rittenberg, 1951) and the plasma proteins one as short as 24 hours, but usually it is longer: 3 days in the rat, 5 in the dog, and 9 to 18 in man (Haurowitz, 1952; Rittenberg, *l.c.*). Uptake by the intestinal mucosa is maximal in an hour (Winnick *et al.*, 1948), but this is for materials merely in transit to the rest of the body; in other tissues also material may remain in transitory states. In the liver, half-lives of eight days are usual, about equal to the life span of a long-lived enzyme molecule (p. 190). In the carcass the uptake of label is still increasing on the seventh day after administration (Sacks, 1953), so that the half life for the whole body must be much longer than this. Nevertheless, it is evidently very short compared with times based on the classical endogenous metabolism.

*In vitro* even simple mixtures of deoxyribonucleoprotein and histone have been shown to incorporate a.a.s (Brunish and Luck, 1952) but incorporation by living cells is seriously reduced when they are merely homogenized, even though this leaves the organelles individually intact; clearly their structural integration is necessary for the full rate of normal incorporation. No organelle is very active in isolation (Pollister, 1954). Incorporation is usually most rapid into the ground cytoplasm (Hultin, 1950; Keller and Zamecnik, 1954) and into the ergastoplasm (Siekevitz and Zamecnik, 1951). The mitochondria supply the necessary respiratory system for the activity (Siekevitz, 1952), and the nucleus maintains the whole system (p. 330). Rupture of the cytoparticles themselves virtually abolishes a.a. incorporation.

Thus biosynthesis of protein is required to replace losses due to accident and wear, even in animals not increasing in mass. It is also required for productive activities and for the work functions. Endogenous metabolism covers all of these activities and is much more extensive than was once supposed. It is possible that metabolic turnover also includes one further component of spontaneous exchange with the fabric, perhaps acting as a margin of safety, and which permits endogenous metabolic processes to tap off more materials if necessary as these flow through the metabolic system. Exogenous metabolism is a smaller fraction of the total than it once appeared to be. It is included in balance sheets of input/output but not in those of metabolic turnover proper, that is of material entering or leaving the fabric of the body.

Turnover rates vary considerably with the organism, the organ, and the material. The range of values agrees with classical estimates of food requirements.

#### **14.3. Formation of the Peptide Bond**

Since this is a condensation or dehydration and not a simple salt linkage (p. 183) it requires considerable energy for formation, and the mode of supplying this has been the major problem in elucidating the natural mechanism. With laboratory facilities Fischer, as early as 1902, synthesized a linear peptide of 18 amino acids, and in recent years oxytocin (oxytocin), bradykinin and other small biological peptides have been synthesized in the laboratory; they prove

to have the properties of the natural molecule and indeed in the case of bradykinin the structure was finally decided from the study of synthetic variants. Fox *et al.* (1959) have synthesized heteropolymers in quantity from a mixture of a.a.s, at temperatures of 160 to 210°C., and even at 70–140°C in the presence of pyrophosphates (including ATP), but these temperatures are outside the range for modern living organisms, even of hot springs. There are reports that simple peptides have been synthesized from ammonium cyanide alone (Lowe *et al.*, 1963) at temperatures as low as 60°C, which is within the vital range for the fauna of hot springs. Most organisms are working at temperatures below 40°C and it is certain that the energy supply for their activities is chemical or photochemical, and not thermal. It has, therefore, been usual to look for enzymic mechanisms of protein synthesis (Borsook, 1953).

Historically, and in conformity with Occam's principle, the first tentative theory of peptide formation *in vivo* was the simplest, namely that proteolytic enzymes are able to work in reverse under appropriate conditions. Phosphorylating enzymes which break down glycogen are capable of working in reverse, at least *in vitro*, and probably *in vivo*. The proteolytic digestive enzymes are known to have specificity for the bonds between particular a.a.s, so that if they could work in reverse they would be able to determine both the general and the specific aspects of peptide synthesis. For the immediate purpose the peptide: H<sub>2</sub>N·(R<sub>1</sub>)CH·CO·NH·(R<sub>2</sub>)CH·CO·NH·(R<sub>3</sub>)CH·CO . . . is being considered as a mere series of peptide bonds though ultimately any specific problems due to the side chains R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, . . . also must be solved (p. 199).

Experiments with the proteases papain and chymotrypsin, using cysteine as an activator (Wasteneys and Borsook, 1930; Fruton, 1950), show some synthesis of an insoluble material, *plastein*, but the amount is limited and is believed to be due mainly to bonds with the  $\omega$ -COOH group of glutamic acid (p. 289), which is not involved in the structure of most natural peptides other than GSH (p. 286). Further, the starting material must be more elaborate than free amino acids, just as these enzymes in their normal capacity do not hydrolyse proteins the whole way to free a.a.s. For synthesis, likewise, the additional help of dipeptidases and other exopeptidases would be anticipated.

In fact, erepsin, a mixture of exopeptidases from the small intestine, will promote some degree of dipeptide formation; moreover, dipeptidases are abundant in embryos and their amount is proportional to the total mass of cytoplasm in the developing embryo (Holter, 1949; Patterson *et al.*, 1948). In the oocyte, again, dipeptidase activity increases at the onset of the formation of yolk protein (p. 128). Heavy metals inhibit plastein synthesis by papain and chymotrypsin, and promote proteolysis, so that a fairly simple switch mechanism for reversing the action might be possible. Again, the pH optima for the two directions are different and might provide a second means of control *in vivo*: proteolysis is optimal at pH 5 and synthesis between pH 7 and 8 (Borsook, 1953).

Later versions of the theory invoked the intracellular proteases, or cathepsins, rather than the specialized externally secreted digestive enzymes, since the synthesis of protein at this level is mainly or entirely intracellular. The cathepsins also are activated by cysteine, and it must be admitted that, in fact, they have considerable resemblance to some of the digestive proteases. There is a correlation between the concentration of cathepsins and protein synthesis in embryos (Holter, 1949; Patterson, 1948; Urbani, 1955), and in regenerating structures (Needham, 1952); in some cases the cathepsin content of a tissue is related to its rate of incorporating a.a.s (Rothschild and Junquiera, 1951).

Hanes *et al.* (1950) found enzymes which resemble typical cathepsins and seemed potentially capable of conjugating peptides of any size. Glutathione and glutamine were transferred in this way to make  $\gamma$ -carboxyl links with a receptor peptide. Moreover, the cathepsins seemed capable of acting as trans-peptidases of this kind (Fruton, 1954). Unfortunately, glutamic acid is  $\alpha$ -linked in natural peptides other than GSH, and no ready interchange of the two links has been demonstrated. Moreover, it was necessary to use unphysiologically high concentrations of reagents, *in vitro*, for this *transpeptidation*.

Jensen *et al.* (1956), Deuchar *et al.* (1957) and Benz and Lehmann (1959) believe that cathepsins are concerned primarily with the regressive, proteolytic phase of regeneration and adequate direct evidence for their synthetic activity has not been obtained. The peptide bond is endergonic, to the extent of 3,500 calories per grammic equivalent of a.a. residues conjugated, and a suitable supply of energy in large packets would be necessary to make these enzymes work efficiently in the synthesis direction. Under normal conditions the equilibrium lies almost 100 per cent in the direction of proteolysis. Including also the necessary energy of activation (Street, 1950), only the molecule of energy-rich compounds such as ATP could supply the required amount per bond. Glycogen breakdown is more easily reversed because the necessary bond energy is retained in the glucose-phosphate residues released (p. 234).

There are other factors which could favour the direction of synthesis, *in vivo*; one is the precipitation of the products. This may account for the modicum of success in synthesizing plastins. Insolubility generally increases with molecular size and it may be largely for this reason that the energy requirement per peptide bond decreases with the length of the growing chain (Borsook, 1953; Linderstrøm-Lang, 1949); the process should, and does (p. 169), eventually become spontaneous. It seems clear that any such facilitation with increasing size should automatically ensure that all new materials are incorporated into the largest existing peptides available, and may account for the paucity of small peptide intermediaries *in vivo* (p. 203). It should also favour the mode of synthesis on templates of existing large molecules (p. 204). The solubility of proteins is further depressed by conjugation with nucleic acids, lipids, polysaccharides (pp. 166, 173) and other substances, and it is significant that in the ergastoplasm protein is associated with both nucleic acid and phospholipid.

Another factor which may decrease the energy requirement is the heterogeneity of natural peptides, just as mixed crystals are formed with less energy consumption than pure ones (Haurowitz, 1950). Certainly this appears to be true for polynucleotide formation (p. 226). A further reasonable speculation here is that systems with a certain combination of heterogeneities of adequate degree may synthesize themselves spontaneously even from their ultimate raw materials, under appropriate conditions, and that it is precisely such autonomous systems which have become what we know as living.

There is something to be learned, which is relevant to peptide synthesis, from the synthesis of industrial polymers, though in general these are homopolymers, resembling the polysaccharides rather than polypeptides. Certainly some a.a.s form homopolymers readily in the laboratory, and glutamic acid, which is outstanding in this respect, also forms polyglutamic sequences in natural biological substances. At the same time glutamic and aspartic acids are outstanding also for their ability to promote the heteropolymerization of a.a.s in general, by Fox's method (p. 193), so that no doubt there are generic aspects common to both types of polymer.

Some industrial polymerizations, once started at high temperature, continue spontaneously at room temperature. This may indicate a decreasing energy requirement per bond with increasing molecular size, just as in peptides. In some cases (Oster, 1954) mild biological reducing agents, such as ascorbic acid (p. 294), will promote industrial polymerizations, a possible parallel to its action in collagen synthesis (p. 294). These same instances of polymerization are accelerated also by the free radicals liberated by light energy in the presence of a photosensitizing dye, and therefore they present some parallel to photosynthesis, the most crucial step of all in biosynthesis. The material itself is oxidized in the process and this may be compared, perhaps, with the oxidation which accompanies the higher grade of polymerization in keratin (p. 173). Endergonic syntheses, such as that of peptides, must be coupled with energy-donating exergonic reactions in order to proceed spontaneously.

There is a theoretical reason for doubting if proteolytic enzymes play a significant part in protein synthesis, even if coupled with a source of adequate energy, and this is the need for strictly irreversible pathways of metabolism (Krebs, 1962) in self-regulating systems such as living organisms (p. 448). Under slight provocation these enzymes would be diverted from synthesis to proteolysis. The search therefore (Fruton, 1954) has been for enzymes which specifically catalyse synthesis, when coupled with an energy-yielding reaction. There has long been evidence that ATP promotes various component reactions of growth, or models of such reactions; examples are the synthesis of the tripeptide glutathione, GSH, by tissue enzymes, the glycine-benzoic link in hippuric acid, and the amide bond in glutamine. The last two are peptide bonds but one component is not an amino acid, and in GSH the glutamic acid is  $\omega$ -linked to the cysteine, though the cysteinyl glycine link is a typical  $\alpha$ -peptide bond. However, little success was obtained experimentally with

amino acids in general and it was a major snag that unlike carbohydrates, purines and pyrimidines, and many other metabolites, a.a.s do not readily form phosphorylated derivatives which could act as intermediaries (Oparin, 1957). In the phosphoproteins the  $\text{PO}_4$  is bonded to the OH of threonine and serine and not to the peptide-forming groups. Acetylation of the a.a.s was envisaged as an alternative, since the acetyl group often is bonded by an energy-rich link, which again could be used to form the definitive peptide bond. Transacetylase does catalyse the formation of some model peptide bonds, but again no good evidence emerged that this could be a major natural method.

An alternative method of reducing the energy requirement is to cover one of the active groups of the free amino acid: otherwise it depresses the facility of the other group to form a peptide link (Fruton, 1954). Covered or substituted compounds such as benzoyltyrosine will then condense spontaneously with others such as glycynamide. However, the model substitutions studied demand special techniques, not known to be relevant to conditions *in vivo*. The search for actual physiological covering groups has been a major aspect of subsequent research.

The search has culminated in the isolation, from various organisms, of what are called amino acid-activating enzymes (Hoagland *et al.*, 1956-58; Zamecnik *et al.*, 1958), which use ATP to cover the COOH group of the a.a. In virtue of the activation energy received from ATP the a.a. then exchanges the latter for a *carrier* or *transfer* molecule of RNA (p. 220), dissolved in the supernatant cytoplasm. This RNA transfers the a.a. to a definitive peptide linkage in a protein on the ribosomes; for this its  $\text{NH}_2$  group is probably used (Chantrenne, 1961). When GSH is synthesized from glutamyl-cysteine and glycine all the enzymes for the three steps appear to be carried by a single protein molecule (Lipmann and Bates, 1961) but this may be a special case. The last three nucleotides, at the active end of all transfer RNA molecules, appear to be cytidylic, cytidylic and adenylic acids respectively, though there may be a different sequence in those transfer molecules which operate in the nucleus (Allfrey *et al.*, 1960).

This mechanism successfully explains activation and other problems, and why ATP and RNA (p. 323) are both so important in protein synthesis. It is also consistent with a template mechanism (p. 204) for the final peptide formation. Moreover nucleic acids themselves are synthesized by a variant of the same sequence of reactions, so that the syntheses of these two groups of key substances may be intimately linked, as work on viruses (p. 153), bacteria (Gale, 1956) and other organisms had already indicated. There is thus good promise that the whole mechanism will show a degree of simplicity at the crowning levels of biosynthesis which a few years ago seemed scarcely possible. Although simple in this light, no doubt the process is complex enough in its entirety, when its control mechanism also is considered.

The first two steps of the process are linked, that is to say the spontaneity of the transfer to the "soluble" RNA depends on the initial activation by ATP.

It is perhaps for this reason that RNA shows ATPase activity in growing yeast (Brachet, 1957, p. 285). Collectively the two reactions are reversible; they may be represented—



where E is the activating enzyme. It is noteworthy that  $\text{Mg}^{++}$  is a cofactor, as usual in reactions involving ATP, that ATP becomes bonded to both substrate and enzyme, and that pyrophosphate is set free, not orthophosphate as in most of its reactions. The high bond energy of the  $\text{aa} \sim \text{AMP}$  conjugate is dissipated in the second reaction, which no doubt largely accounts for its spontaneity. The mitochondria are normally necessary for the process, in cell-free preparations, but are dispensable if extraneous ATP is added (Loftfield, 1957). Their function, therefore, is to maintain the continual resynthesis of ATP through coupling with oxidation-reduction reactions.

Guanosine triphosphate, GTP, the analogue of ATP (p. 226), seems to be specifically required for the third step, the transfer of a.a. from aa. RNA to the final peptide linkage. Details of this stage are uncertain, though a linear condensation is favoured (Loftfield, 1957; Commoner, 1959; Dintzis, 1961) rather than simultaneous marshalling of all the a.a.s required for a particular molecule. These and other alternatives will be considered in the next section. There is evidence that each a.a. may have its own specific activating enzyme but this does not necessarily decide between the two alternatives. It should be a valuable aid to the construction of specific, as opposed to random, a.a. sequences, though for reasons given later it could not dispense with the need for a specific model or template for each protein.

The carrier RNA molecule also is probably different for each amino acid, but the carrier for a particular a.a. is very similar in different species of animal (Holley *et al.*, 1960). Further requirements for the subsequent transfer to peptide linkage include cysteine or GSH, a large, heat-labile molecule, presumably a protein (Nathans, 1960) and the potassium ion. For protein synthesis in the nucleus there appears to be much the same mechanism as in the cytoplasm, except that the sodium ion is required in place of  $\text{K}^+$  in this particular role (Allfrey *et al.*, 1960).

In the synthesis of nucleic acid by this system the essential reaction is—



where NTP is the triphosphate of any of the nuclear nucleosides (p. 226), including ATP itself. Again PP is set free and the bond, NMP · RNA, is energy-poor, like that of aa · RNA; it is already a polynucleotide bond, in fact, and the RNA molecule has grown by one nucleotide residue. Free nucleoside di-phosphates also will conjugate in this way since the second P

also is energy-rich bonded; orthophosphate is set free in this case. It seems likely that *in vivo* there may be a balance between the demands of a.a.s and nucleotide phosphates for sites on the RNA carriers, or on the ribosomes and this alone may be an important factor in the mutual control of synthesis by proteins and nucleic acids.

The a.a.-activating (a.a.a.) system is even more versatile than this. It can activate other amino acyl compounds, acetyl and other acyl groups, monose phosphate, choline phosphate and sulphhydryl (SH) groups. All are activated by conjugation with ATP, and with the release of PP and are then transferred to further, definitive combinations. This, therefore, seems to be the key to many pathways of biosynthesis, and to other metabolic pathways. It is to be distinguished from another large and important group of actions by ATP (p. 318), resulting in phosphorylation of the substrate and liberation of the AMP (adenosine monophosphate) or ADP (Lipmann, 1958).

In bacteria other enzyme systems have been discovered, which may prove to be either adjuncts of, or alternatives to, the a.a.a. system. One, called the "incorporation enzyme" system (Chantrenne, 1961), is independent of the a.a.a. system and causes the triphosphate of each of the common nucleosides (p. 220) to transfer its terminal P, probably direct to an a.a., forming the kind of active phosphorylated a.a. so long sought (p. 196). The a.a.s can then be bonded into a peptide. A single species of a.a. and one species of tri-P nucleoside can produce a di-, tri- or tetra-peptide (Monod, 1962) while a mixture of species of both can build quite large heteropeptides. There is a molecule of orthophosphate liberated for each peptide bond formed and this bonding seems to be via the COOH, and not the NH<sub>2</sub> group as suggested for the a.a.a. system. Every a.a. is active with at least one NTP, and vice versa, but each enzyme is specific to one of the NTPs and only four different enzymes are required to synthesize polypeptides in general. Gale (1959b) discovered "incorporation factors" in staphylococci which appear to be nucleoside derivatives and therefore may be related to this system.

A number of further factors have been found to play a part in protein synthesis. One of these is Sachs's soluble "S-protein" which promotes the incorporation of a.a.s into the microsomal particles (Chantrenne, 1961). A lipid incorporation factor (Butler *et al.*, 1961), probably a lipoprotein, containing phosphorus, has been recognized by Hendler (1958) and may be concerned with the final stages on the ribosomes (Chantrenne, 1961), after transfer by RNA. The ribosomes are held to the endoplasmic membranes by lipid, which is dissolved away by deoxycholate and may correspond to the lipid cement of some viruses (p. 153). It is also possible that carbohydrate is attached to the lipid (Chantrenne, *i.c.*, p. 111), and Gale (1961) finds glycerol related to his incorporation factor. Glycerol may be significant as a component of glycerides, or still more of phosphatides, though there is the further possibility that it provides further evidence of the parallel between muscular contraction and protein synthesis, since the relaxing factor may prove to be glyceraldehyde.

In any event, members of virtually every main class of biological material appear to be involved in protein synthesis.

In all, a great deal is now known about the natural method of forging peptide bonds, and the subject is rapidly becoming as well understood as catabolic processes. The major problem is inevitably the way in which the synthesis is organized. The large number of biological materials involved and the great versatility of the a.a.a. enzyme systems provide strong evidence that protein synthesis is the focal point of biosynthesis.

#### 14.4. The Synthesis of Specific Heteropeptides

The conjugation of a.a.s in a specific sequence is a more formidable problem than the supply of energy to forge peptide bonds at random. von Bertalanffy (1960), using Boltzmann's principle, calculates that a specific order adds 2 kilocalories per mole of bonds to the 7 kcal for random union. It is necessary, therefore, first to be sure that the sequence really is absolutely specific in each protein. In general, proteins do seem to be taxonomically specific, and even peculiar to the individual animal. They differ between organs of the same body, and even within one cell or body fluid there are a number of clearly characterized proteins. The physical and other gross properties of any one protein, as well as its immune reactions, are consistent, and its gross amino acid composition at least is constant (Fromageot, 1949), but *a priori* this could be purely statistical, with no rigid spatial pattern (Pirie, 1937; Fox and Homeyer, 1955). Even the purest of protein preparations in fact show some heterogeneity in electrophoretic behaviour, and in some cases at least a whole family of related molecular types appears to be synthesized. The strong dependence of synthesis on a simultaneous presentation of all the necessary a.a.s in particular ratios (Haurowitz, 1952) again might imply no more than statistical consistency. Changes in diet result in changes in the gross a.a. composition of the body (Cohen, 1959), but this is simply due to differential rates of synthesis of the different specific proteins. Those proteins which have been analysed for their a.a. sequence (Sanger, 1952) indicate a very specific order, and it is generally thought that most proteins may be considered each a single molecular species (Campbell and Work, 1953).

The problem of specific ordering would be relatively simple if the structure of all proteins conformed to mere permutations of some one or more simple patterns of a.a.s. The simplest of patterns, provided it involved a substantial number of the common a.a.s, could provide at least as many different proteins as are found in all living organisms. Some proteins such as cytochrome, collagen and silk contain only a few a.a.s and these almost certainly are arranged in a very simple pattern, with a short repeat period, but these proteins are as exceptional as they are limited in variety. It is not so certain that proteins with a richer a.a. composition have these arranged in relatively simple familial patterns, though some evidence of this kind of simplicity has been obtained. At one time it seemed possible that proteins rather generally had molecular weights which

were multiples of a value around 17,000, which would be equivalent to 288 amino acid residues (Svedberg, 1937). Bergmann and Niemann (1937) found that the number of residues in the proteins they analysed could be expressed by the general formula  $2^m \times 3^n$ , where the integers  $m, n$  are specific to each

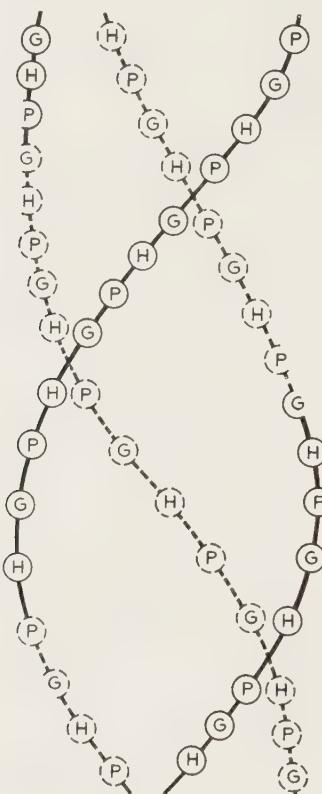


FIG. 14.1. DIAGRAM TO ILLUSTRATE THE STRUCTURE OF THE PROTOFIBRIL OF COLLAGEN, A CABLE OF THREE HELICALLY CO-COILED POLYPEPTIDE CHAINS  
Each chain is a simple regular repetition of the amino acids proline (P), hydroxyproline (H), and glycine (G). The chains are adjusted so that all three different amino acids occur at any transverse section of the cable. The diagram illustrates: (1) one of the simplest sequences of amino acids in a peptide, (2) the fibrous type of protein macromolecule, (3) one type of tertiary structure in proteins (cf. the myoglobin type (p. 207)), (4) the coiled-coil structure—each polypeptide chain individually is a first-order spiral.

(After A. Rich)

protein. This could mean that a.a.s, irrespective of their identity, unite in small groups of two or three residues, which in turn link in two's or threes with other units of the same size, at each level of magnitude. This would be essentially polymerization in geometric progression. The 288 residues of Svedberg's unit give the formula  $2^5 \times 3^2$ . The same rule was found to apply

to the number of residues of each particular a.a. However, there has not been general support for Svedberg's rule (Pedersen, 1949) and only limited support for that of Bergmann and Niemann (Astbury, 1943); they may apply only to the special cases such as that of collagen (Fig. 14.1), qualitatively poor in a.a.s, which are arranged with a simple and very regular repeat period (Astbury, 1940; Rich and Crick, 1955). A geometric mode of polymerization could ensure a simple specific order of a.a.s, though it need not necessarily do so. Leach and Lindley (1952) detected a lower grade of regularity in the composition of a functionally related group of proteins, all enzymes. The ratio of the amount of the dicarboxylic amino acids, glutamic and aspartic acids, to that of the hydroxy acids, threonine and serine, was near unity and there were other regularities. The implication is an orderly arrangement, but one less simple than those mentioned above. Once more, however, the regularity might be statistical only.

In insulin (Sanger, 1952) and other proteins the pattern has proved far from simple, though quite specific. Many a.a. sequences occur only once, though certain particular dipeptides are repeated and other pairs are consistently one residue apart. Cysteine is two residues removed from proline in several places in the molecule. The molecule is, therefore, to a high degree aperiodic, and Gamow *et al.* (1956) could find no significant deviation from a random order, in the sense of an order devoid of coincidences, in the number of proteins so far analysed. It may be pointed out that randomness in fact includes some coincidences and that it is necessary to test if the actual number is significantly less frequent than by chance.

The order is specific and constant in each protein, however, and so the very antithesis of random in this sense. The mammalian posterior pituitary hormones, oxytocin and vasopressin, ideally simple proteins, containing only eight a.a.s, differ in two of these, in an otherwise identical sequence. Both hormones vary in a similar way taxonomically among the mammals. Fairly simple differences are found also between the various factors of the anterior pituitary, which have larger molecules. The haemoglobin of those human beings with the sickle cell trait differs from the normal in only one of the 288 a.a. residues, and other forms of Hb have been found, with similar small differences. Thus different proteins in the same body, and the same protein in related animals, differ only in details of a very specific sequence.

While the simple patterns of such proteins as collagen, and those implied by the Bergmann-Niemann rule would be consistent with a mode of synthesis by geometric progression, the highly aperiodic molecules would seem to demand copying on an unique template or jig. It need not be cast simultaneously throughout its length and Dalgleish (1953, 1957), Loftfield (1957) and others have envisaged a linear process of accretion on a template (Fig. 14.2). Equally the linear, arithmetic mode of growth might occur without any jig or template, if there were some other device for specifying the a.a. sequence. There is, of course, no guarantee that all proteins are synthesized by the same method,

and the possible roles of linear, geometric and template modes will be considered in turn.

Plasteins (p. 193) appear to grow by linear accretion, and the enzyme which catalyses the condensation of glycine with glutamyl-cysteine will not condense glycine with free cysteine (Snoke and Rothman, 1951), possibly implying an obligatory linear sequence, which will always cover one active group of one participating a.a. (p. 196). The action of exopeptidases is by linear attrition. Simple linear accretion occurs in the biosynthesis of such homopolymers as the polysaccharides. The existence of a.a. carriers (p. 196) seems most consistent

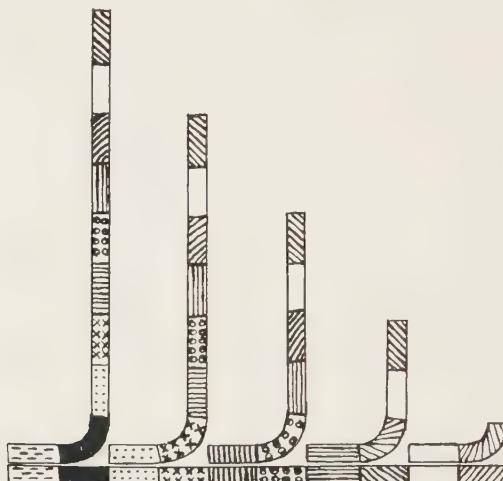


FIG. 14.2. DIAGRAM TO ILLUSTRATE DALGLEISH'S THEORY OF SIMULTANEOUS SERIAL TEMPLATING

Five molecular copies are in process of formation on the template molecule below. Each is synthesized progressively, and immediately peels off from the relevant section of the template, allowing another copy to follow in its wake. At any one moment the equivalent of one complete copy molecule is on the template.

with an arithmetic mode of cooperation, though it does not necessarily demand this. It could be slow by comparison with the other two methods, though there might be a place for slow synthesis. Claude (1949) envisaged the method as keeping many a.a.s hanging about, awaiting their turn for assembly. However, Dalgleish (1953) has pointed out that there could be a close sequence of linearly synthesized molecules simultaneously peeling off from a template, each attached at one point only (Fig. 14.2). The equivalent of one complete molecule would then be formed at any one moment, the maximal rate for any template. While this is essentially a template theory it is evident that the same phenomenon would be possible without templates; provided there were an adequate number of molecules in all stages of construction no a.a.s need be idle. Actual cases where there is strong probability of linear accretion have

been described by Commoner (1959), Dintzis (1961), Gerber and Altman (1961) and Friedrich-Freksa (1961), among others.

A geometric method of growth of protein molecules (Syngle, 1943) might be possible only in the supernatant, where the condensing fragments of all sizes are free to move, and does not seem compatible with a template mechanism. It would be a possible method not only for the production of the Bergmann-Niemann patterns but for synthesis by transpeptidation (p. 194). The endopeptidases break down proteins into large fragments which, reciprocally, might be the penultimate stage of synthesis, even though the enzymes may not be the same. If the method does occur then intermediary peptides should be detectable, and there is some evidence of these. Small peptides have been identified in blood, urine and elsewhere in the body (Dent, 1950; Sanger, 1950), and a greater increase in oligopeptides than in either free a.a.s or completed proteins was found in the growing bean root (Morgan and Reith, 1954). Growing bacteria liberate oligopeptides into the medium (Gale, 1953; Borsook, 1953) and penicillin may inhibit some stage intermediate between free a.a.s and completed protein (Gale, l.c.). The incorporation into ovalbumin of labelled aspartic acid, presented for a very short interval, was found to be very patchy (Steinberg and Anfinsen, 1952) as though it has been incorporated into particular hexapeptide moieties which later condensed with unlabelled oligopeptides. Others found uniform labelling under these conditions, indicating simultaneous synthesis of the whole molecule. It must be appreciated that unless the labelled a.a. is applied very briefly, and the products isolated at the appropriate moment, there will be statistically uniform labelling whatever the mode of synthesis. One result showing patchy distribution might be more significant than many indicating uniform labelling (Meister, 1959). Opinion is very much divided but it inclines against the widespread occurrence of peptide intermediaries (Ebert, 1954a; Brachet, 1957). To some extent the problem may be complicated by the occurrence of definitive oligopeptides in the body, for instance GSH, the posterior pituitary hormones, bradykinin, some antibiotics and other substances.

There are two further criteria for detecting this method of synthesis: amino acids should be incorporated into oligopeptides more readily than into larger molecules and oligopeptides should be more rapidly incorporated than free a.a.s into the latter. In fact cysteine is more rapidly incorporated into GSH than into larger peptides (Anderson and Mosher, 1951), but GSH is a special and definitive peptide. Some cells have been found to use peptides, even of foreign origin, more effectively than they use free a.a.s (Fischer, 1946; Francis and Winnick, 1953; Jackson, 1953; Randall, 1953). It seems doubtful if foreign oligopeptides could have enough correct a.a. sequences to be used more efficiently and rapidly than free a.a.s but the evidence about indigenous peptides is more impressive. Even so it seems that most tissues (Borsook, 1953) and bacteria (Gale, 1953) prefer free a.a.s, and this conclusion continues to be supported strongly.

A geometric pattern of synthesis of molecules does not necessarily demand a geometric rate, and reciprocally a geometric rate is compatible with, if not demanded by, *any* mechanism in which the products of growth themselves continue to grow (p. 11). Again, a geometric rate at the molecular level does not necessarily cause a geometric rate at the cell level, if other factors have effective control over cell division (p. 338).

Although template, or "cookie pusher," mechanisms (Spiegelman and Sussman, 1952) may seem rather naïve, it is difficult to envisage a reasonable alternative for the continued synthesis of a genetically specific and unique protein (Chantrenne, 1952a; Loftfield, 1957); detailed and consistent information must be continuously available. At a somewhat higher level of magnitude the chromosomes (p. 135) multiply by just such a copying process, and this may be true of some other cell organelles (p. 140). There was some criticism of the early forms of the theory because they invoked intermediary negative replicas (Haldane, 1937) which would be wasteful, but in fact the same kind of criticism could be levelled at heterotemplates, which now seem the most plausible. Wastage is not serious if the mould can be used repeatedly, as it is in industrial practice.

At one time (Delbrück, 1941) a direct positive templating seemed probable, depending on the property of mesomeric resonance which proteins, among other organic molecules, possess. Mesomeric molecules have properties intermediate between two alternative tautomeric forms between which they may be considered to oscillate in dynamic equilibrium. Such molecules might be able to attract their identities over considerable distances (Haas, 1937), and identical a.a.s therefore might be martrialled alongside the peptide template and then conjugated together. The heat of formation of a mesomeric molecule is lower than either of the forms between which it oscillates, and the difference, the resonance energy, might be available for promoting peptide-bond formation between the a.a.s of the copy, or for stabilizing these bonds once formed. The phenomenon might also help in the exchange of individual a.a.s (p. 188). Unfortunately there is considerable doubt if resonance could provide significant forces and energy for the purpose (Stockmeyer, 1959; Kasha, 1959). The discovery of the a.a.-activating systems moreover has made the problem of energy supply less critical.

It has also strengthened the alternative view that proteins are built on a heterotemplate of nucleic acid. A heterotemplate has the virtue that it can never be so directly autosynthetic as a protein template, and so it is presumably less liable to uncontrolled excess. Hinshelwood (1956) has stressed the danger of simple autocatalysis, and the evidence that in fact no individual component of the living material is autocatalytic in isolation. The possible exception is DNA, the construction of which (Watson and Crick, 1953) as pairs of complementary polynucleotide strands seems designed specially for self-copying. The belief that DNA or RNA, or both, might act also as heterotemplates for protein synthesis (Haurowitz, 1950; Dounce, 1952; Gamow, 1954; Gamow

*et al.*, 1956) is based on much circumstantial evidence (p. 323), and there have been a number of ingenious attempts to elucidate the type of code by which nucleic acids could specify peptides. Certain critical intervals along the polynucleotide chain fit very tolerably other critical distances on the polypeptide chain, which could be accommodated in the groove between the two strands of the typical DNA duplex (Wilkins, 1956). It has therefore been speculated that the constellation of two or three nucleotides nearest to each a.a. on the peptide under synthesis might absolutely specify the nature of that a.a. (Gamow *et al.*, 1956). The four different nucleotides of DNA (p. 220) could provide twenty different non-overlapping constellations of three nucleotides, which is just adequate for the common structural a.a.s.

Crick and his colleagues (Crick *et al.*, 1961) have now collected considerable evidence in favour of the code which in general has seemed the most plausible, a non-overlapping triplet code. Moreover, in the bacteriophage which they studied it is always "read" by the synthesizing system from one particular end, so that there can be no confusion. However, they are led to conclude that the code is imprecise, or "degenerate," that is to say, several triplet combinations of nucleotides can specify the same a.a. This may largely explain the great variation among the accumulating body of results purporting to show which nucleotides actually specify each a.a. in particular organisms (Lengyel *et al.*, 1961; Martin *et al.*, 1961-2). However, even if there is a degenerate coding one should still obtain a reasonable structure for the hypothetical coding RNA molecule by applying the a.a. codes to a protein of known a.a. sequence. Using this technique, however, and working back from the known a.a. sequence in bovine pancreatic ribonuclease, Chargaff (1962) finds that the RNA required to specify it would have very bizarre ratios of its four bases.

The actual nucleotide-a.a. specificities which are being revealed are of course established fact and not dependent on any particular theory, so that it should soon be possible to define the code within limits from the knowledge of these alone. In general, they show only that a particular a.a. needs a certain nucleotide, and not necessarily what other nucleotides are required for complete specification, nor their order.

Among other types of code which have been suggested the one which is considered most plausible at present (Chantrenne, 1961, p. 118) is a two-digit code, instead of the four digits of the four nucleotide bases. It depends on the presence of an NH<sub>2</sub> group or of a double-bonded =O group at position 6, each of which occurs in two of the nucleotide bases. This code has the virtue of being based on chemical properties, and also that of great simplicity. Notwithstanding this it could specify the necessary number of a.a.s, by using groups of five nucleotides. Some people may feel that it is approaching too closely the sophistication of the digital computer systems to be biologically plausible.

Now that the a.a. sequence is known for several large protein molecules it is possible to work out for one the possible polynucleotide codes, and then to test these against other proteins. No type of code has yet been found which

is valid for more than the one protein, but this may mean that the correct code has not yet been broken, and not that the general theory is wrong. It is possible that different codes operate in different organisms and even between proteins of the same body, though the high degree of biochemical uniformity in most fields discourages this view. Whatever the code there seems little doubt of the reality of the general NA-protein type of heterotemplate mechanism. The growth of bacteriophages (p. 153) definitely, and of other viruses probably, involves an alternating reciprocally catalysed synthesis of protein and NA, while the nucleoprotein structure of ribosomes and chromosomes in all organisms provides circumstantial evidence that the mechanism is universal.

Both proteins and nucleic acids frequently form spirally coiled molecular threads or co-coiled bundles of threads (Pauling and Corey, 1953), so that templating, and still more the subsequent separation from the template, present serious spatial problems (Delbrück, 1954), similar to those for daughter chromatids (p. 136). The spiral structure is effectively three-dimensional so that the simple two dimensional type of template used industrially is inapplicable, and for the same reason it is not necessary to insist that the site of synthesis must be a biological membrane. In the cytoplasm it is the ribosomes rather than their supporting membranes which appear to provide the templates.

Templates are likely to prevent fortuitous changes in the a.a. sequence of the product proteins and contribute greatly to the maintenance of genetic control. The present evidence (p. 326) is that the template material, carrying the genetic specifications, is renewed very frequently so that if the gene itself remains stable so do its messenger templates and its ultimate products. A mutational change in any particular template molecule will have a trivial effect on the total population of product molecules because of its short life, and so would any inexact copying of a template. Significant somatic mutations are changes affecting the nuclear gene, or sometimes, perhaps, a self-reproducing cytoplasmic body. There is, of course, the further possibility that occasionally a gene consistently produces inexact templates, but this would probably be indistinguishable from a mutational change.

The general conclusion from this section is that proteins with a unique and complex a.a. sequence, the majority of proteins in fact, are synthesized on a template and this is a heterotemplate of nucleic acid. The code which transcribes the one into the other is not yet certain but there is much positive evidence in favour of a non-overlapping nucleotide triplet code for each a.a. The assembly of a.a.s on the coded template is likely to be in a spiral linear order but the problem of the subsequent separation of the completed protein from the template is still unsolved. The union of two or more peptide fragments, which would have to occur free in the cell sap, is probably rare at this level of size, though common at the level considered in the previous chapter.

The union of free peptides at that level is mainly a homopolymerization of similar peptide units and it therefore seems a reasonable generalization that templates are required only at the stages of specifying the a.a. sequence in these

units. There are a number of interesting intermediate structures, also. For instance, the haemoglobin molecule consists of two pairs of peptide units, each synthesized on its own ribosome site, while adenylosuccinase may be a compound of nine different peptides of this kind. The subsequent union of the four haemoglobin sub-units is probably spontaneous at normal pH (Halvorson, 1960) and so resembles the simpler polymerizations and may likewise occur free in the cell sap.

Another intermediate stage of which there is as yet little real knowledge is the tertiary folding of peptides (Fig. 14.3) to form globular proteins (Haurowitz,

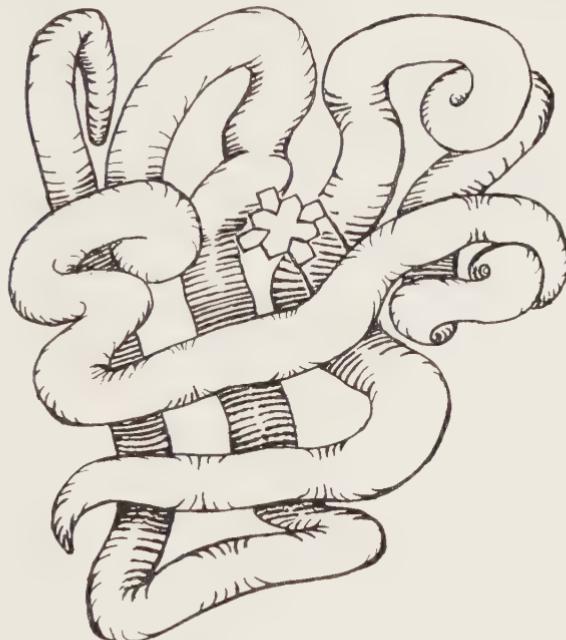


FIG. 14.3. DIAGRAM OF THE MOLECULE OF MYOGLOBIN, TO SHOW THE IRREGULAR MODE OF FOLDING OF THE SINGLE, UNBRANCHED PEPTIDE CHAIN TO FORM A TYPICAL GLOBULAR TERTIARY STRUCTURE

The attachment of the haem prosthetic group is indicated.

(Based on Kendrew, 1959, 1961)

1950). Straub *et al.* (1961) are led to suggest that an RNA fraction controls this so that it may approximate to the template type of process: the bonds are probably more specific than those in free polymerizations.

#### 14.5. The Synthesis of Enzymes

Technically enzymes are particularly valuable proteins for the study of synthesis, since they are effectively labelled by their activity: the increase in their activity is a measure of their rate of synthesis. Some enzymes, such as

cytochrome and catalase, have, in addition, characteristic absorption spectra due to their prosthetic groups. Moreover, like all proteins, enzymes can be estimated as antigens (Monod and Cohn, 1952). The study of enzyme synthesis has contributed very materially to the general knowledge of protein synthesis, as well as to that in other fields.

Enzymes are very sensitive to the level of nitrogen intake so that starvation usually leads to a prompt fall in activity, and therefore in amount of the enzyme, particularly in the cytoplasm (Laird and Barton, 1954; Litwack *et al.*, 1950; Awapara, 1953). The most indispensable enzymes appear to be the most resistant to this atrophy. Enzymes concerned with protein metabolism itself often show special properties: thus arginase, which is concerned with the disposal of nitrogen from excess amino acids, increases indefinitely with the amount of protein ingested, whereas general growth does not increase any further once protein exceeds 33 per cent of the total diet (Anon, 1953a).

For probably all substrates which are invariably present in, or presented to, the body there exist constitutive enzymes, the activity of which changes little under conditions other than starvation. More useful for the study of protein synthesis is the so-called inducible or "adaptive" type of enzyme which is synthesized only to meet the emergency of having to use some less common substrate, and which atrophies again on withholding this (Monod and Cohn, 1952; Spiegelman, 1950). In fact synthesis of the enzyme ceases abruptly when the substrate is exhausted or withheld (Pardee, 1962) and the induction may be equally prompt, so that enzyme is detectable within a few minutes of adding a new substrate (Halvorson, 1960). Sometimes there is a preinduction period, which possibly may be compared to the lag periods in the growth cycle of viruses, in the induction of antibodies, and in other growth processes, but it appears that the response has all the efficiency, economy and other features of a good biological adaptation. The original term, "adaptive enzyme," emphasized this but it is less satisfactory than *induced enzyme* since a constitutive enzyme is a biological adaptation of at least equal importance.

Enzyme induction has been studied mainly in microorganisms and it is here that the study offers richest dividends. In the higher Metazoa hormonal and other systemic agents complicate the picture (Knox *et al.*, 1956). There is good evidence, however, that the phenomenon occurs in all organisms (Chantrenne, 1961).

As a process of protein synthesis enzyme-induction appears to be typical in most essentials, and it has added greatly to our knowledge of this process. For instance, it shows that there is a general principle of economy in biosynthesis and that this is manifested in a competition between pathways of synthesis for available nitrogen. If there is an abundant supply of suitable nitrogenous materials then induced enzymes persist longer than usual after their inducing substrate has been withdrawn or exhausted. In the competition for N the maintenance of the constitutive enzymes at their optimal level has priority over the synthesis of induced enzymes and this is further evidence of biological

adaptation. Among induced enzymes themselves competition is most severe between those catalysing reactions in the same metabolic pathway. This has been taken as evidence that these enzymes themselves are structurally related and are competing for a common precursor "proteinogen" or "pre-enzyme" (Halvorson, 1960), different from the precursors of the enzymes for other pathways. However, there is another reason why competition is particularly severe between these enzymes and this is that they must be induced at the same time if the substrate is to be metabolized effectively. This collective induction of all the enzymes for a complete pathway is one of the outstanding features of the phenomenon (Spiegelman, 1956).

Precursor proteins which could be quickly "finished" to any one of a whole family of definitive proteins (Northrop, 1949) could help to simplify biosynthesis, and in principle should greatly accelerate the response to sudden demands for new enzymes, antibodies (p. 214) and other proteins. On the other hand the potential rate of protein synthesis *ab initio*, from free a.a.s is quite adequate and there is as yet no very good evidence for the proteinogen kind of precursor. In the case of induced enzymes there is very strong evidence that synthesis is in fact mainly, if not entirely, from free a.a.s (Spiegelman, 1953). It is generally agreed that existing mature proteins cannot be remodelled for another purpose, though they may be completely hydrolysed to a.a.s and reused. For enzyme synthesis, as for growth in general, dietary sources must contain all the essential a.a.s (Hopkins, 1951), those which the organism cannot synthesize itself, whereas the non-essential a.a.s are not critical. Indeed sources of N such as simple ammonium salts, in the diet, promote the synthesis and maintenance of enzymes as effectively as this group (Litwack *et al.*, 1953).

There is an increase in the RNA content of cells which are synthesizing an induced enzyme (Spiegelman, 1950), and this RNA also is synthesized from its ultimate units, the purine and pyrimidine bases (Gale, 1956; King, 1959). This is to be expected if the heterotemplate theory is correct, since a new protein will demand a new type of NA template. Constitutive enzymes on the other hand are adequately maintained by their existing RNA templates. The induction of  $\beta$ -galactosidase is inhibited by ultraviolet light of the wave-length most strongly absorbed by NA, and most effective in destroying it (Chantrenne, 1952a). Spiegelman (1953) thought that ribonucleoprotein might be acting in a special capacity as a specific "inductin" for the synthesis of the enzyme but it seems probable that the RNA is performing its usual role in protein synthesis. At the same time no doubt it is quite specific to the particular enzyme induced (Halvorson, 1960). Like the protein itself this RNA is not formed by remodelling other, mature species of NA.

The early stages of induction, like the early stages of virus multiplication, are particularly sensitive to inhibition by irradiation (Chantrenne, 1961). In addition, however, enzyme induction seems to be a particularly sensitive biosynthetic process, and is the first to suffer in any emergency. In autotrophes it is more sensitive to respiratory inhibitors than, for instance, the initial fixation

of carbon and nitrogen into organic form. It is more sensitive than cell division to temperature changes (Knox, 1953). It is certainly much more sensitive than the subsequent action of the enzyme to most environmental agents.

Like protein synthesis in general (p. 196) enzyme induction is coupled with the oxidative synthesis of ATP as the general mediator of high-energy transfers. The process is therefore inhibited by respiratory poisons such as azide and dinitrophenol. However, some enzymes are induced under anaerobic conditions, and they are, in general, enzymes which subsequently function under these conditions. This implies that there is some direct relationship between induction and the reaction catalysed by the enzyme, a possibility which might have been anticipated from the fact that the substrate for this reaction is the normal inducer. It is further significant that substances related to the normal substrate also are quite good inducers.

This raises the whole question of the mechanism of induction, which to some extent may be peculiar to this one process, but, on the other hand, is relevant also to the synthesis of constitutive enzymes, and may prove to have wider significance in biosynthesis. Induction itself is strictly speaking part of the control of biosynthesis, and could have been dealt with in Part II, but at the molecular level it becomes increasingly difficult to divorce control from the actual process, and it is most conveniently considered here.

It would be anticipated that induction by the actual substrate might be a particularly simple and direct mechanism. Yudkin (1938) described a simple and instructive chemical model, namely that in order to dehydrogenate formic acid, palladium must be prepared from its nitrate by the action specifically of formic acid. The induction of an enzyme could scarcely be as simple as this but the enzyme might be formed from a precursor by a reaction with the substrate which was simply the preliminary to the definitive reaction itself. The substrate might activate the precursor in much the same way as reciprocally the enzyme normally activates it, by forming a complex with it. This once more opens the case for some kind of ready-made precursor, and the observed neoformation of protein and NA from their ultimate units might be evoked to replenish the precursor as it is used. This might be a simple mass-action effect (Yudkin, 1938), so that further synthesis would cease as soon as the precursor pool was refilled and no longer being tapped.

Unfortunately enzyme induction proves to be much more complex than this, the superficial simplicity being the culmination of a process of natural selection for a simple "objective," by means which are devious (Needham, 1937). It is biologically desirable that sporadically available substrates should evoke appropriate enzymes when, and only while, they are presented, but the proximate causation is not simple. Apart from that already given there is not much evidence that induction is at all closely associated with the subsequent catalysis by the enzyme, and on the other hand there are a number of sharp distinctions between the two, in addition to the greater sensitivity of induction to respiratory inhibitors and other environmental agents. For instance, boron

inhibits the induction of  $\beta$ -galactosidase but not its subsequent action. The pH optima of the two differ: maltozymase is synthesized most rapidly at a pH greater than 8.5, which inhibits the actual fermentation of maltose. This and other carbohydrates when acting as inducing substrates do not even serve as a source of energy for the synthesis of their enzymes; glucose is virtually always used for this purpose, even after enough of the enzyme has been induced to open up the new pathway. Oxygen is required only for the initial stages of enzyme induction, even in cases where it is continuously required for the subsequent catalytic action. In fact, the substrate itself, as inducer, is not required after the initial *preinduction* period, the so-called Pollock effect, so that it is extremely unlikely that induction is a preliminary to the catalytic reaction. Some inducing agents are quite unrelated to the normal substrate.

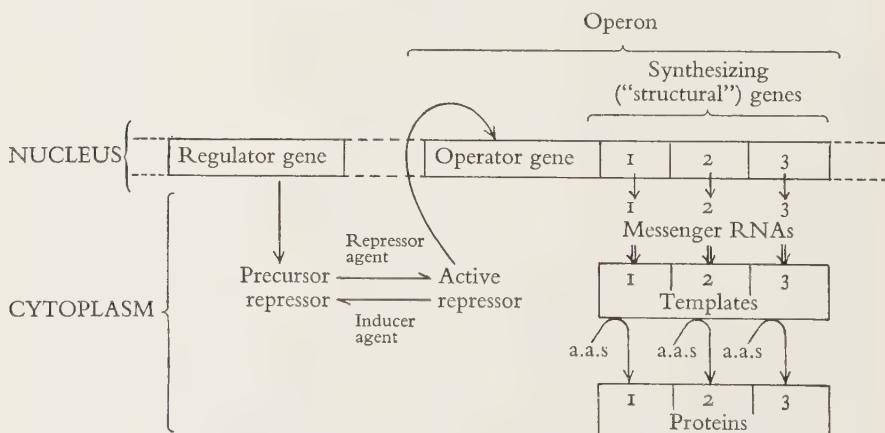
Nevertheless a chemically specific process is involved, as shown by the fact that analogues and derivatives of the substrate are tolerably good substitutes. Those inducing agents which bear no close chemical affinity to the normal substrate presumably have some particular, active radical in common with it and in some cases the nature of this has been discovered (Monod *et al.*, 1951). The different inducers of a particular enzyme act at the same site (Landman, 1953) and induce an identical protein. Of course, *isodynamic* enzymes, that is enzymes catalysing the same reaction in different organisms, have different protein moieties, a fact of no little interest in the question of homology (De Beer, 1938).

The precise site of the inducing action is still very uncertain (Jacob and Monod, 1962; Pardee, 1962), though the Pollock effect seems to imply that a conjugate of some kind is formed, so that it remains when the free substrate is removed. The conjugant could even be the postulated enzyme precursor, since the only certain evidence is that the complex can induce the synthesis of more of the active enzyme. Provided that it could be induced without being permanently conjugated with substrate the initial conjugate could continue its work indefinitely. However, there is no compelling reason for advocating this direct conjugate and on the other hand there is now much evidence that the action is more indirect.

The whole investigation took a new turn with the discovery that induction is not in fact an activation process but the removal of inhibition (Hogness, 1959). In *Escherichia coli* a dominant gene *i*<sup>+</sup> was discovered which gives the individual the ability to synthesize  $\beta$ -galactosidase when induced. In the presence of the recessive *i*<sup>-</sup>, however, the bacterium produced the enzyme at all times, that is constitutively, so that the inducible phenotype depends on the possession of a dominant repressor or regulator gene, which controls a second gene, *z*, responsible for the synthesis of the enzyme. In some way inducing agents block the action of this regulator gene and allow the "structural" gene, *z*, to function. At the same time it is noteworthy that enzymes can be induced in enucleate halves of *Acetabularia* (Picken, 1960, p. 142), so that the action is on cytoplasmic representatives of the genes.

It is generally agreed (Jacob and Monod, 1962; Pardee, 1962) that the regulator gene must act through the cytoplasm, or at least through a deputy of some kind, on the synthesizing gene if it is to be susceptible to such environmental agents as inducing substrates. At present a direct action on the gene *in situ* is not ruled out but it is improbable in the light of these facts and of knowledge of genetic mechanisms in general. The theory of Jacob and Monod is that the regulator gene does work by producing a repressor substance which is passed into the cytoplasm. There is also evidence of substances which antagonize the effects of inducers and these are thought to activate the hypothetical repressor substance while inducers block this activation step.

Knowledge of the genetics of the mechanism has developed apace and there are further complications, but here we are interested only in the outlines of its epigenetics. Briefly the further complication is the one already mentioned (p. 209), namely that all the enzymes for the complete pathway of metabolism of a new substrate must be induced in concert. It was originally thought that the product of each step in this pathway would act as inducer for the enzyme catalysing the next step, so that the whole suite would follow automatically from the switching on or off of the first step, but it is now clear that the whole mechanism would become extremely complex if each enzyme had its own separate regulator gene and repressor. In addition there is positive evidence that the synthesizing genes are functionally linked, affected collectively by mutations in the one regulator gene, or in one other gene, the *operator*, which exercises a promotor action on them all. The regulator is thought to act on them through the operator. Often they are all close together on the same cistron (p. 390), with the operator gene nearby, the whole set being termed the *operon*. The complete mechanism therefore might be represented as follows—



The mode of action of the operator is perhaps the most obscure aspect, particularly in cases where the synthesizing genes are not contiguous but scattered through the genome (Gorini *et al.*, 1962). Maas (1962) suggests that inducing

agents promote, and repressors prevent, the release of enzyme from the site of its synthesis on the ribosomes, so that the idea of a latent enzyme precursor might again be in court. However, this would require either a separate repressor for each of the protein enzymes of the pathway or one which was non-specific and would therefore affect enzymes in other pathways. Action through the operator seems a necessary provision. For further details and discussion the reader is referred to Volume 26 of the *Cold Spring Harbor Symposia on Quantitative Biology*.

It seems probable that some of the competition mentioned earlier (p. 208) may be due to repression. For instance, Monod (1958) has shown that when two alternative substrates are presented simultaneously to an organism it metabolizes one completely before attacking the other, a phenomenon which he calls *diauxie*. The enzymes for the second substrate are temporarily repressed. Constitutive pathways repress inducible ones, just as they compete successfully with them. It is evident that potentially the whole course of metabolism may be a selective system based on this kind of interaction. The potential synthetic powers of the body are vastly greater (p. 11) than those normally realized (Cohen, 1959) so that repression or some kind of inhibition certainly plays a major role. The scheme of Jacob and Monod visualizes every controlling action as an inhibitory one. This is also likely to be the most effective type of control in living organisms (p. 425).

There are many further manifestations of metabolic switches and adjustments (Davis and Yudkin, 1952), which are probably based either on a mechanism of enzyme induction/repression or on that of activation/suppression to be considered later (p. 319). Many microorganisms are able to switch between oxidative and reductive pathways of deamination of amino acids, according to the pH of the medium (Gale and Epps, 1942; Carter and Thompson, 1953). By doing this they vary the amount of acid produced and so adjust the pH back to neutrality. There is an inherent relationship between rH and pH, that is to say redox and pH phenomena are not independent so that again the mechanism of adjustment superficially might appear to be a simple and direct chemical process. The specificity of active amino acid oxidases varies with the particular a.a. presented (Edlbacher, 1946). In facultative anaerobes even the cytochrome system is inducible (Aubel, 1959).

More obscure phenomena which may prove to be even greater refinements of the enzyme induction mechanism have been discovered, for instance by Davis (Horowitz and Leupold, 1951) and Lundegårdh (1950). Davis found that when an enzyme was bound, and its action inhibited, by an analogue of the normal substrate, it gradually changed its character in favour of greater affinity for the latter and so there was considerable recovery of the normal rate of metabolism. Rather similarly Lundegårdh found that when the cytochrome of plant roots was blocked by such respiratory poisons as cyanide and azide it gradually recovered its ability to transfer electrons and to promote the absorption of anions by the root. In both cases we may possibly envisage the synthesis

of an enzyme more absolutely specific to the normal substrate and more indifferent to the analogue. Supposing that the molecules of a particular enzyme do vary somewhat in properties, there could conceivably be a shift in the modal type synthesized. A simple competitive-selective mechanism at the level of the catalytic reaction by the enzyme might be adequate since those enzyme molecules with greatest affinity for the normal substrate would be able to perform their normal reaction and would continue "in circulation" while those with greatest affinity for the blocking substrate would be immobilized. The mechanism which normally controls the amount of enzyme synthesized might be expected to permit more to be synthesized, until there were enough molecules of enzyme with a selectively high affinity for the normal substrate—and enough of those with a selectively low affinity to bind the substrate analogue.

The value of inducible/repressible enzymes to catabolize sporadic sources of energy is evident enough, but in the case of synthesizing enzymes it might be expected that all would prove constitutive, since the nature of the essential fabric of the body is genetically fixed, and much the same irrespective of diet. However, certain building materials may be present ready-made in some diets and not in others; a useful economy can be effected by suppressing the enzymes concerned in synthesizing these particular materials in the first event and inducing them in the second. Moreover, the fabric itself is not absolutely invariant; pigments and other materials vary with the nature of the environment, with age, season and so on. A number of anabolic enzymes have been found inducible, for instance those for the synthesis of nicotinic acid and its nucleotide derivatives (Koser and Wright, 1943) and for thiamine synthesis (Wood *et al.*, 1938). The enzyme for incorporating tryptophan into proteins disappears when the organism is starved of tryptophan (Borsook and Deasy, 1951).

The incorporation of amino acids, and the synthesis of coenzymes such as thiamine and nicotinic acid are required for the synthesis of enzymes themselves; in the induction of anabolic enzymes, therefore, we glimpse the kind of vicious circle (Davis, 1962) which is one of the major problems of growth: the difficulty Kingsley appreciated, of explaining how certain key components make themselves. An indirect autosynthesis is implied here and conforms to the requirement of Hinshelwood (1956). The more indirect the pathway and the greater the number of other metabolites involved the less chance there is of any one being synthesized in disproportionate amount.

Enzyme induction is a valuable way of studying protein synthesis and at least as much work has been done with this end in view as with the more special objective of the induction mechanism itself. The latter is an outstanding example of intracellular control mechanisms and has the added importance that its genetic basis is being rapidly clarified.

#### 14.6. The Synthesis of Antibodies

Antibodies (a.b.s) like enzymes, carry a good functional label in addition to the common properties by which all proteins are recognized. Also, like

inducible enzymes, they are synthesized on demand; in this case they are induced by *antigens* (a.g.s), that is to say harmful foreign proteins, or other foreign materials associated with protein. They are functionally specific to the inducing a.g., with which they combine, rendering it innocuous; their molecules are probably also structurally specific to the antigen, therefore. Taking all these features into consideration, their importance in the study of protein synthesis is scarcely less than their special physiological interest to the immunologist. This special interest cannot be considered here; the reader may be referred to Boyd (1956), Sevag (1951), Burnet (1956, 1959), Najjar (1959) and other works.

Antigens enter the body in a number of ways and may become distributed widely via the blood stream. However, they are taken up selectively by the liver, kidney, bone marrow, spleen, lymph nodes and phagocytes of the host. This has been demonstrated very clearly by the use of fluorescent a.g.s (Coons, 1951). The organs mainly involved are therefore the excretory organs, and the organs which produce blood cells and a.b.s, so that the body's first response seems to be to localize the antigen where it can be dealt with most effectively, first as a toxic chemical and secondly as a specific a.g. Antibodies begin to appear in the lymph nodes, spleen and bone marrow after an induction period which may be as short as 30 minutes but is usually longer. They are released into the circulation and contribute to the  $\gamma$ -globulin fraction of the plasma. The peak concentration is reached in four to six days and then begins to fall, as the a.g. is progressively neutralized and eliminated. Antibodies persist, for a time which varies enormously in the different cases, from 20 days to a lifetime. *A priori* it would be supposed that a long persistence, to meet the risk of further experiences of the a.g., would always be valuable and it is therefore possible that there are counteracting advantages in the short persistence in certain cases. It may be significant that there is only a brief persistence of the a.b. against the common cold, which is not very lethal.

It is possible to prepare fluorescent a.g.s. (Coons, 1951), so that they can then be traced in a host body by fluorescence microscopy. They accumulate first on the small particles of the cytoplasm and later on the mitochondria (Haurowitz, 1959), in the same way as the raw materials for protein synthesis; they may influence a.b. protein synthesis in some very direct way, therefore—for instance by speeding, or by causing some other modification of the transcription process on the RNP templates of the ribosomes. There is just the possibility that the selective accumulation on the cytoplasmic particles is diagnostic of fluorescent substances in general rather than of a.g.s specifically (Elevitch and Brunston, 1961), and the behaviour of chemical carcinogens, which are often fluorescent (p. 106), is relevant here. In any case it is interesting that the uptake of fluorescent a.g.s by the particles is inhibited if these are liberated from the cell by homogenizing it. The normal structural organization of the cell therefore is essential.

On theoretical grounds (Schweet and Owen, 1957) it is often considered

that a.g.s must actually enter the nucleus and alter the pattern of protein synthesis at its initiation in the genome. The assumption is that the latter then sends a new type of messenger RNA (p. 325) to the ribosomes with instructions to synthesize a new a.b. protein, specific to the a.g. The idea is very similar to one of the theories of carcinogenesis (p. 99) but, as in that case also, there is as yet no good evidence that the nucleus is usually violated. Viruses change their host's pattern of synthesis very radically (p. 156) though only a few of them enter the nucleus. They may of course influence the genome more indirectly and may affect the nucleus without entering it. Antigens promote cell proliferation in the host and so do many viruses, while carcinogens also do so eventually; this must be ultimately an action on the nucleus.

A direct and directional effect on the instructions sent out from the genome would be effectively a directed mutation and would be viewed very critically by geneticists. It also implies that environmental factors rather easily affect the genome and this also would be treated with scepticism on present evidence.

Antibodies are probably synthesized inside mobile cells which circulate in the blood stream and which can be recognized by the very high concentration of ribonucleoproteins in their cytoplasm. They stain intensely with pyronin in consequence (Wissler *et al.*, 1957). They are generally thought to belong to the group of blood cells known as plasma cells (Burnet, 1959; Humphrey, 1960; McMaster, 1961), having a common origin with lymphocytes. Wissler believes that they originate from reticulo-endothelial cells but in any case they circulate repeatedly between blood, lymphoid tissue and lymph stream (Gowans, 1959), probably acquiring a.g., or delivering a.b.—or both.

They increase in number after a challenge by an a.g., in parallel with the rise in a.b. titre of the blood, and this is smaller when their rate of proliferation is depressed by X-rays, by starvation or by special a.b. inhibitors; the immunological reaction by the host is depressed proportionately (Wissler *et al.*, 1957). Their numbers decline sharply once the amount of a.b. in the blood has passed its maximum. Patients suffering from lack of  $\gamma$ -globulins in their blood (agammaglobulinaemia) also lack plasma cells and have a poor defence against a.g.s in general (Burnet, 1959). Pieces of spleen and lymph node containing plasma cells continue to synthesize a.b.s *in vitro*, though a.b.s against new a.g.s cannot be induced here (Humphrey, 1960). Each cell produces between 100 and 1,000 molecules of a.b.

There is usually a lag, or induction, period after a challenge, before any a.b. can be detected. This recalls the similar period in many cases of cell proliferation, in virus multiplication and in enzyme induction. On this and other grounds Burnet (1959) and Szilard (1960) have developed in detail the view that a.b. induction is closely similar to enzyme induction. Of course this does not necessarily imply that the subsequent reaction between a.b. and a.g. is enzymic. In fact it appears to be a simple stoichiometric reaction, forming a stable complex. Very often more than one a.b. molecule is required for each one of the a.g.

If spleen tissue is introduced from an already immunized individual there is

no lag so that this probably depends on the time normally taken by an a.g. to stimulate the spleen and other poietic tissues to produce sufficient active plasma cells. Some event which occurs during this time is particularly sensitive to inhibition by irradiation (Taliaferro, 1957), just as in the multiplication cycle of viruses and in the induction of enzymes. There is little doubt that this event is likewise concerned with nucleic acid synthesis, and the damage due to irradiation can be rectified by administering enzymatic hydrolysates of either RNA or DNA. This nucleic acid presumably is used either for the new template which determines the synthesis of the specific a.b. or for the genetic material which determines the template itself, and it is significant that the NA hydrolysates promote a.b. production much better in the presence of a.g. (Taliaferro, l.c.). The latter therefore may influence the specific structure or the properties of the a.b. in some way, as most theories maintain, though it might be acting simply as a general stimulus to synthetic activity.

There is further support for the view that the action is on an early, template-forming stage in the fact that a.b. production continues after all detectable a.g. has disappeared from the body. This seems very comparable to the Pollock effect in enzyme induction (p. 211). In the light of the present interpretation of enzyme induction (p. 212) the possibility must be envisaged that the action is at an even earlier stage than that of template formation by the synthesizing gene. In the case of virus multiplication, however, the counterpart of this phenomenon definitely is concerned with the stage of template formation.

Little is yet known about the actual synthesis of the a.b. molecule but it is now certain (Chantrenne, 1961) that once more free a.a.s are used, and not mature proteins, or even large peptide precursors (Taliaferro, 1957). The  $\gamma$ -globulins of the plasma are closely related to a.b.s in all their major diagnostic properties but if they are suitably labelled and an a.g. then given to the animal no label appears in the a.b. subsequently produced. On the other hand labelled a.a.s are rapidly incorporated (Chantrenne, l.c.). It therefore appears that the  $\gamma$ -globulins are derived from antibodies and not vice versa: possibly all the blood  $\gamma$ -globulin is antibody material (McMaster, 1961). In the young mammal the amount of  $\gamma$ -globulin which was received from the mother decreases to a minimum at 2-4 weeks after birth and then increases again, in parallel with the beginning of plasma cell production by the infant (McMaster, l.c.).

There is little more that can be said with certainty about the synthesis of antibodies or about the way each is specified, but it is a growth phenomenon too striking and too important to leave without a little more exploratory speculation. A question of considerable importance is: can an animal produce an a.b. against any a.g. which it might encounter? This could include all the proteins of every other living organism; few other substances act as a.g.s unless they are conjugated with protein but even such simple ones as inorganic elements and tartaric acid (Chantrenne, 1961) become antigenic if they are conjugated in this way. Each conjugate acts as a different a.g. from the unconjugated protein so that the total number of possible a.g.s might be legion.

However, not all foreign proteins prove to be potent a.g.s, and perhaps only those which adversely affect the host's metabolism evoke the a.b. response. This may be just a special part of the detoxication mechanisms of the host, therefore. In support of this idea is the fact (p. 215) that many a.g.s are extensively destroyed in the body's major excretory organs, the liver and the kidney.

The a.b. response therefore is not omnipotent. Moreover, it is not always functionally adequate even in cases where a response is induced. Sometimes the animal suffers from a violent deleterious reaction known as allergy or anaphylaxis. This reaction is not usually serious at the first challenge by any a.g. but it rapidly augments on subsequent occasions and is sometimes fatal. Whatever the full explanation of anaphylaxis it is clear that the animal's defence is not perfect. Sometimes the a.b.-a.g. complex itself acts as a new antigen (Najjar and Robinson, 1959) and conceivably this may be the basis for anaphylaxis, particularly if each generation of a.b.-a.g. complexes in turn acts as a further a.g.

Again the degree of specificity of the a.b. to the a.g. should not be overstressed, though it remains impressive enough. Sometimes the animal produces a whole range of different protein molecules as its a.b. to a particular a.g., and virtually always an a.b. cross-reacts with proteins related to the a.g. which induce it. The strength of the cross-reaction is fairly closely proportional to the degree of taxonomic affinity between the donors of the antigenic proteins and has been used in deciding affinities, and in confirming other taxonomic evidence (Boyd, 1948; Boyden, 1943, 1953). It does more to confirm than to deny the specificity of the a.b.-a.g. relationship and indicates that the basis for this is chemical. It is also compatible with the view that a.g.s are simply substances that happen to react critically with one of the host's proteins: the a.b. may be only a variant of this protein so that the necessary genetic instructions for its synthesis are already present.

In some cases an a.b. has been found to react with a protein having no recognized taxonomic affinity to the initial a.g. (Chantrenne, 1961) but this also is consistent with a chemical basis for specificity. Occasional chemical resemblances between taxonomically disparate proteins are to be expected among the large total number. It is also possible that those properties of proteins which are significant for antigenic purposes are not so numerous and varied as the differences in amino acid composition and sequence, so that in consequence fortuitous antigenic resemblances are not excessively rare.

It has been a common view that an animal's protein-synthesizing mechanism dances to whatever tune may be called by foreign a.g.s and the a.b.s induced are very precisely complementary in structure to their antigens, after the manner of heterotemplates (p. 204)—or of a lock and key, to use Ehrlich's classical analogy. In fact, however, antibodies seem to be more host-specific than antigen-specific. For instance nine different a.b.s induced in the rabbit, four against pneumococci and five against ovalbumin, were all found to have the same five a.a.s, in the same sequence starting from one end of the molecule: alanine, leucine, valine, aspartic acid and glutamic acid. In four others only the

terminal a.a. was identified but this was again alanine (Chantrenne, 1961). The a.a. composition of all was the same within the limits of experimental error and they were indistinguishable except by immunological properties (Humphrey, 1960). There is scope for variation even among proteins having the same gross a.a. composition, but in any case the animal defends itself on its own terms, using protein patterns which are not unlimited.

In this event the mechanism may indeed resemble orthodox detoxication fairly closely, allowing for the fact that the chemical complexity and specificity of proteins are greater than those of most other foreign substances encountered. Antibodies then may detoxicate a.g.s by attacking their dangerous chemical groups by means of certain standard groups, in the same way as say glucuronic acid detoxicates a large number of organic substances (R. T. Williams, 1959). In this way a relatively standard pattern of a.b. might deal with a great taxonomic variety of a.g.s. However, it is almost certain that a typical foreign protein has a number of active toxic groups, that is to say groups capable of disturbing the host's metabolism, so that a combination of a number of standard antibody groups would be required to deal with them, and the number may be great enough to account for the observed degree of specificity between a.b. and a.g. At the same time all the a.b.s of one animal may belong to a restricted group of its proteins, contrasting with the variety of a.g. molecules they can inactivate.

Because of its degree of specificity a particular antibody is made only to order, whereas glucuronic acid is always in stock and always in use against a number of toxic substances. Many of the latter are completely inactivated when glucuronic acid reacts with a single critical radical and there is a close parallel to this in the vulnerability of antigens.

This idea still leaves the growth problem unsolved: how does an a.g. induce the production of a specific a.b.? Whether the synthesis is entirely new or is simply a selective acceleration of the synthesis of one particular type of stock molecule it is still as remarkable as it is obscure. Enzyme induction is rather more prosaic since it is not particularly surprising that organisms should have latent powers of dealing with fairly common and simple substrates when these become available. Among the mysteries of immunology is the phenomenon of tolerance to a.g.s if these are presented early in life (Billingham, 1959). It is easy to see the biological value of this, in preventing antibody reactions against the new proteins which have come together in the zygote, but the mechanism underlying it is as obscure as positive antibody induction.

Antibodies therefore are specific proteins synthesized in response to the challenge of dangerous foreign protein materials. They selectively combine with these and effectively inactivate them, except in the curious pathological condition of allergy. They are synthesized from free a.a.s, through the growth and proliferation of plasma cells and the synthesis is associated with that of NA. Synthesis of a.b. often continues after all the a.g. has been destroyed and leaves a reserve of a.b. to prime the response to a subsequent challenge. The mechanism of induction and production of a specific a.b. protein remains very obscure, however.

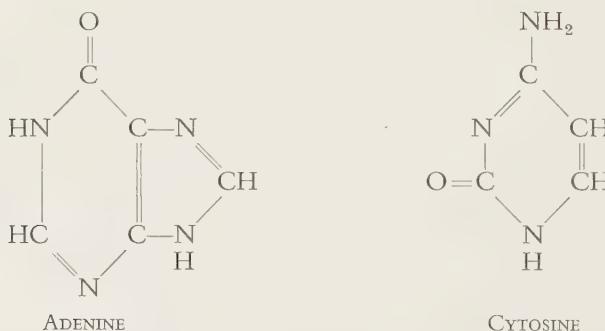
## CHAPTER 15

# *The Biosynthesis of Nucleic Acids, Nucleoproteins and Other Biological Substances*

For the reasons already given (p. 183) the nucleic acids are the only remaining group of materials which will be considered in any detail. Only points of major interest in the biosynthesis of other substances will be raised and these scarcely merit a separate chapter.

### 15.1 Nucleic Acids

In view of the outstanding role of nucleic acids in growth their own synthesis must be considered briefly; in any case it is itself an interesting example of molecular growth. The structure of the nucleic acids is now fairly well known (Davidson, 1957; Chargaff and Davidson, 1955). They are linear heteropolymers of units called nucleotides, each consisting of a purine or a pyrimidine base linked via a pentose sugar residue to phosphoric acid, which also provides the further link for polymerization. Except in transfer-RNA



(p. 196) only two purines, adenine and guanine, and three pyrimidines, thymine, cytosine and uracil are common, so that there are only one-quarter as many common nucleotides as amino acids. The base-sugar compounds in isolation, adenosine, guanosine, thymidine, cytidine and uridine are known as nucleosides, and the complete nucleotide therefore is known alternatively as adenosine monophosphate, AMP, or adenylic acid, and so on.

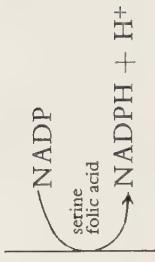
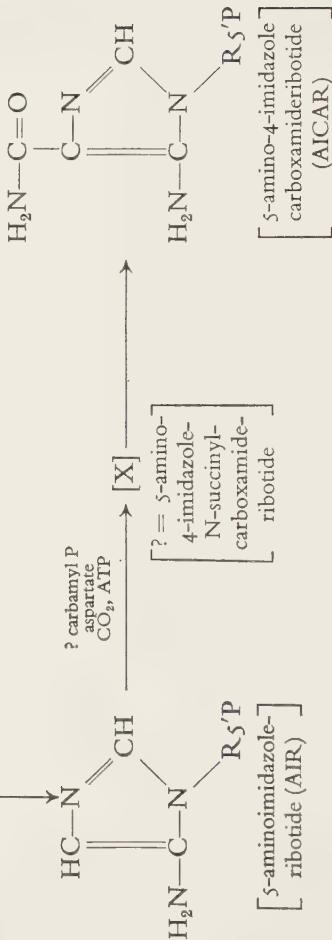
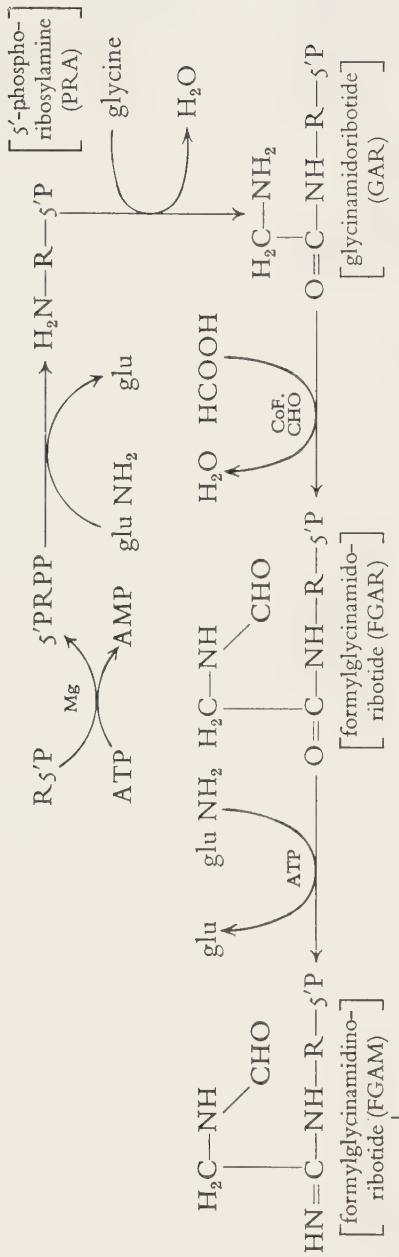
There are two main forms of nucleic acid, distinct in structure, properties and distribution. Deoxyribonucleic acid, DNA, with deoxyribose (p. 225) as its sugar, is largely restricted to the chromosomes, while RNA or ribonucleic

acid, is most abundant in the cytoplasm and the nucleolus. DNA contains no uracil and RNA no thymine, but the other three bases are common to both. DNA forms the larger polymers, with molecular weights up to two million Daltons. Usually two complementary molecular threads are wound together (p. 226) and they then assume a regular spiral structure. RNA molecules may exist as single molecular chains and this often has no regular spiral form, so that on X-ray examination they do not show the same orderly or crystalline structure as DNA.

Even heterotrophes are able not only to form nucleotides from their three components, obtained in the diet, but also to synthesize the bases themselves. They use dietary sources of these as far as available, however, and devices have been detected (Magasanik, 1958) which depress the more basic synthesis when possible, in favour of using these. The probable pathways of synthesis of the bases (Figs. 15.1, 15.2) are outlined by Welch (1956) and others, and they illustrate some important general, as well as specific, features of biosynthesis. Thus the synthesis is a fundamental one, from simple precursors: amino acids,  $\text{CO}_2$  and small radicals such as  $-\text{CHO}$  and  $-\text{NH}_2$ . Secondly, it does not proceed via what seem the obvious structural intermediaries, urea and triose sugar, as classical theory rather naturally supposed; further it is not the direct reverse of the known pathway of catabolism, for that of the purines first yields the imidazole derivative, allantoin, with a fully saturated imidazole ring. Again the synthesis pathway for the pyrimidines is not identical with the first part of the purine pathway although structurally the pyrimidine ring is a component of the completed purine molecule. In purine synthesis the imidazole ring is formed first. Nevertheless this imidazole derivative is not common to histidine synthesis as might have been expected. There is a single pathway for the two purines, however, and one for the three pyrimidines, that is to say within each group the bases are extensively interconvertible.

A further point of general interest is that adenine and guanine control their own synthesis by a negative feedback device; this is probably part of the mechanism for exploiting dietary supplies of the bases when available. It also controls the subsequent step, that of nucleotide synthesis, by mass action. Another interesting feature is that the purine bases are formed already conjugated with ribose phosphate; this is another example (p. 5) of components which play a part both in the synthesis and in the subsequent functioning of a molecule.

It helps to explain the speed and relative spontaneity of synthesis—why it runs to completion in one piece, as it were. Here and in many pathways of biosynthesis large amounts of intermediaries rarely accumulate unless blocking agents are used. However, the points of punctuation, that is the intermediaries which do accumulate, vary in different organisms; the nucleotide stage is the usual point, but in the anthers of the lily deoxynucleosides accumulate at one stage, whereas the subsequent nucleotide stage seems to be transient (Stern, 1960). Again the precursor of the pyrimidine bases, unlike that of the purines, is



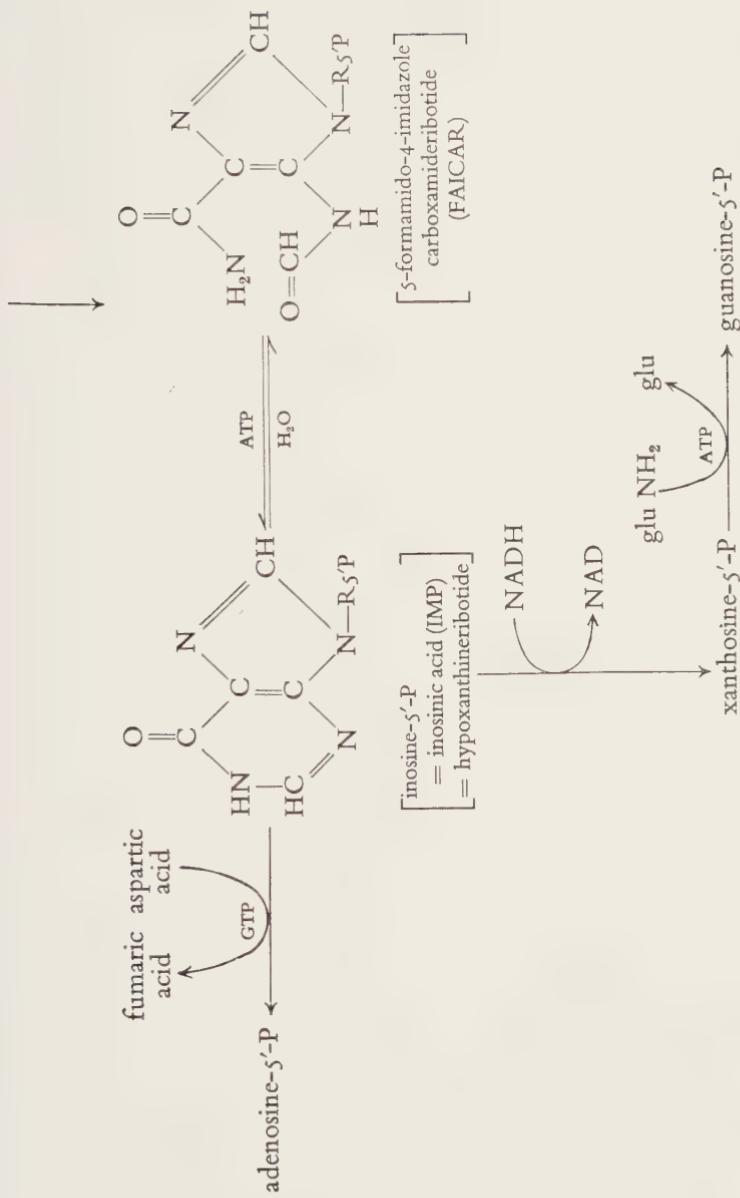


FIG. 15.1. PATHWAY OF BIOSYNTHESIS OF PURINES

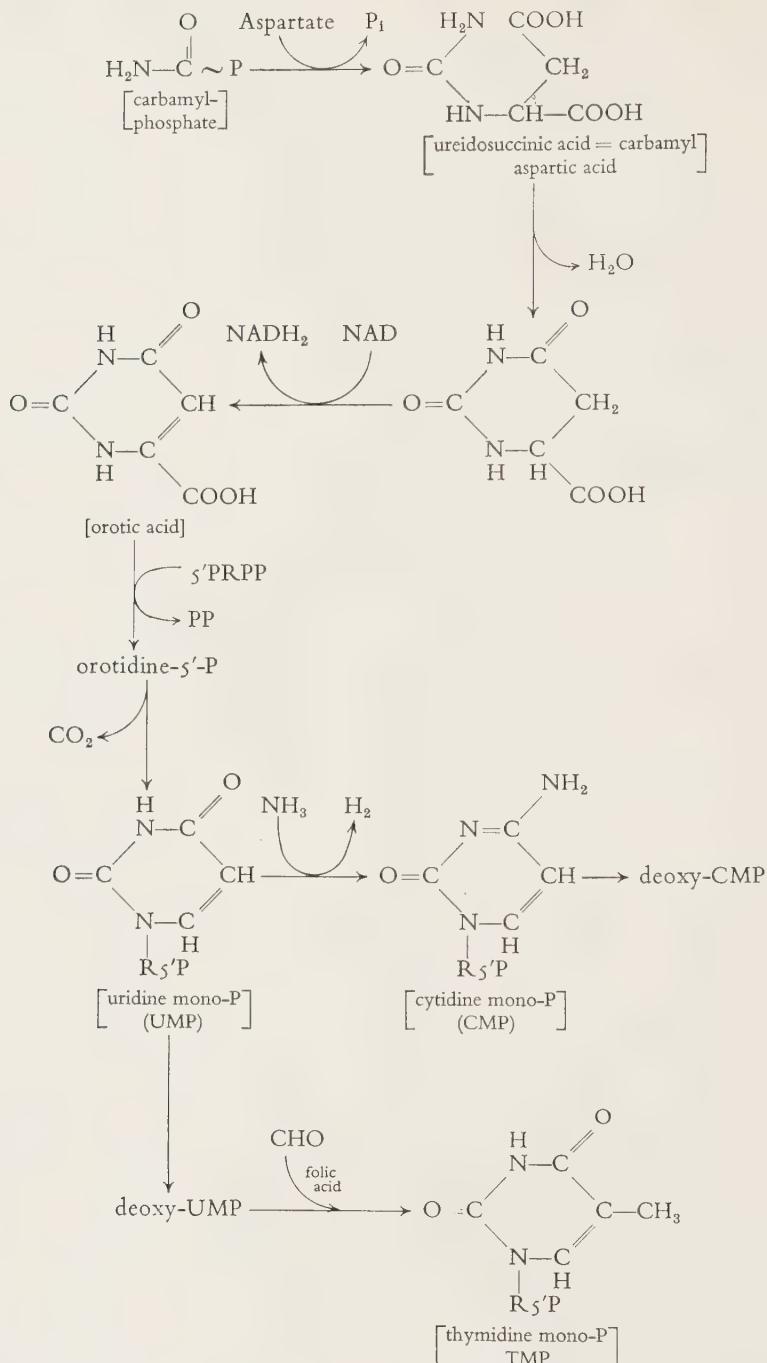


FIG. 15.2. PATHWAY OF BIOSYNTHESIS OF PYRIMIDINES

formed free but it can then be converted to a nucleotide at one step by reaction with the important metabolite PRPP, 1'-pyrophosphoryl-ribose-5'-P.

This compound in fact is the starting point for the synthesis of the purines, so that it is a key metabolite in the pathways of both groups of nucleotides, but at different points. It is formed from ribose-5'-P by pyrophosphorylation with ATP. In animals ribose itself, D-ribofuranose—



is mainly formed from glucose via the gluconic acid shunt pathway (p. 259), and then is ready phosphorylated. The deoxyribose of DNA—



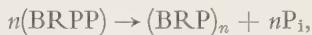
may be formed by condensation between glyceraldehyde-3-P and acetaldehyde (Florkin, 1960), but at present it seems more probable that ribose derivatives are converted by reduction to their deoxy counterparts at higher levels of the pathway of synthesis (Cohen, 1959). Rather similarly the cytosine and thymine derivatives are formed from the initial pyrimidine, uracil, at the nucleotide level; this may help to explain why few organisms can incorporate free pyrimidine bases extensively, though the nucleosides are phosphorylated and used. Extraneous purines are utilized as free bases as well as in completed nucleotides (Brown, 1951). Free bases are converted to nucleosides by ribose-1'-P and not by the 5'-P; the nucleoside is then phosphorylated to nucleotide by ATP.

The B-vitamins, nicotinamide and riboflavin, form nucleotides by very similar reactions, but do not subsequently polymerize in the same way as the nuclear nucleotides (p. 226). Riboflavin is already a nucleoside analogue, having the alcohol ribitol in place of the aldehyde ribose, and so it is phosphorylated by ATP to give flavin mononucleotide. Nicotinamide probably forms its mononucleoside as above, by transribosidation from ribose-1'-P (Baldwin, 1953, p. 354); the ribose is then re-phosphorylated at the 5'-position by ATP. By generally similar reactions a number of the other B-vitamins form phosphorylated derivatives, but most of them not containing ribose. In metabolism they usually behave like the true nucleotides in taking on further phosphate residues in high-energy pyrophosphate linkage (symbolized  $\sim$ ), and becoming coenzymes of fundamental transferases (p. 303). ATP pyrophosphorylates a number of these in a single step, itself being degraded to AMP. The nicotinamide and flavin mononucleotides conjugate with a further molecule of ATP to form dinucleotides linked by their phosphate radicals. Both the mono- and dinucleotides form coenzymes of various transhydrogenases (p. 307). In forming the dinucleotides pyrophosphate is set free, so that the conjugation

compares with the various "activation" reactions (p. 196). Phosphopantetheine forms coenzyme A (p. 313) by this same reaction.

Like adenylic acid, the other nuclear nucleotides form free di- and triphosphates, also with coenzymic and other metabolic functions. They are formed in the same way as ATP from the nucleotide, by coupling with oxidation reactions, but usually with ATP itself as the P-donor; it seems that AMP and ADP are unique in their ability to take on  $P_i$ , by respiratory coupling. As coenzymes the nucleotide triphosphates promote a number of reactions, mainly anabolic and, as tested *in vitro*, they can deputize for ATP to some extent in promoting certain phases of the muscle contraction cycle. *In vitro*, and probably *in vivo*, they act as ready-energized building bricks for polymerization to nucleic acids. In addition, as already noted (p. 196), they all, but ATP and GTP in particular, have specific functions in protein synthesis.

There is some similarity between the polymerizations of natural nucleotides and those of amino acids. In the laboratory both can build homopolymers, but more readily form heteropolymers, like the natural products. Enzymes capable of catalysing extensive homo- and heteropolymerizations of four of the nucleoside diphosphates, ADP, GDP, CDP, and UDP, have been extracted from *Azotobacter* by Ochoa (Warner, 1957), and quantities of the mono-, di-, and triphosphates of all four have been found in the cells. The action of these polynucleotide phosphorylases may be represented—



where B is any of the bases.

GDP gives a poor yield of homopolymer but readily heteropolymerizes with the other diphosphates. In equimolar amounts the four diphosphates give polynucleotides with the properties of the RNA of the species from which the enzyme came. Here, therefore, the enzyme protein specifies the nucleic acid, in accordance with the reciprocal heterotemplate hypothesis (p. 228). The linkage is as in natural RNA, between the 3'-OH of one ribose residue and the 5'-OH of the next, via the phosphate group. This may be contrasted to the —P—P— linkage of the nicotinamide and flavin dinucleotides.

Poly-UMP and poly-AMP will spontaneously aggregate with each other stoichiometrically, to give a stable structure; the units of poly-AMP are ten times the size of those of poly-UMP so that they aggregate in the ratio of one to ten. The order of these units seems to be random, however. The linkage is by hydrogen bonds, which are the main bonds in polynucleotide and polypeptide associations generally, essentially lateral bonds between molecular chains. The poly-AMP-poly-UMP association is a two-stranded helix like natural DNA, and it is possible to add a third, polyuridine, strand to this (Rich, 1959); this facility may be taken as an indication that the templating of NA molecules on existing ones is relatively spontaneous. Most mixed nucleotide polymers form single strands, however, like natural RNA. The relation between this system and the system of Hoagland and Zamecnik (p. 196) is not yet clear but since it

usually also demands a full range of free a.a.s (Chantrenne, 1961; Niedhardt and Fraenkel, 1962) it is probably an actual component of the "activating" system. At the same time it is possible that the different RNA fractions of the cell—nucleolar, messenger, microsomal and transfer (soluble) RNAs—are not all synthesized by the same method. Spiegelman found a system in the membranes of *E. coli* which requires nucleoside triphosphates, and Mn as cofactor (Gale, 1959a), in contrast to the diphosphates and the Mg cofactor of Ochoa.

Kornberg (1957, 1960) extracted from *Escherichia coli* a similar enzyme system capable of forming DNA, using thymidine di- and tri-P in place of UDP and UTP. An ATP-kinase for initially pyrophosphorylating the nucleotides also can be extracted from this bacterium. Again heteropolymers are formed more easily than pure polymers: indeed all four nucleotide polyphosphates must be present for good activity. Polymerization demands the presence of a *primer*, or starter, amount of DNA, and the higher energy of the tri-Ps as compared with di-Ps is advantageous, if not indispensable. There appear to be no restrictions on the linear order of nucleotides, and all sixteen possible pairs of neighbours occur.

In *E. coli* the DNA priming is necessary also for the synthesis of some of the RNA fractions (Gale, 1959a; Hurwitz *et al.*, 1962). In the presence of a simple polymer of thymidylic acid (TMP), adenylic acid likewise builds up only a homopolymer, even in the presence of the other deoxyribonucleotides. Using simple copolymers of TMP and deoxyadenylic acid (DAMP) as primers the ribotides build an adenylic-uridylic polymer, with the two monomers regularly alternating. Formed RNA, by contrast, is not necessary to prime this synthesis, but it may be essential for the synthesis of microsomal RNA, since this is inhibited by the enzyme ribonuclease (RNAase).

The way in which DNA acts as its own template was indicated by the classical work of Watson and Crick (1953), showing that DNA normally exists as a helix of two co-coiled molecules, with their purine and pyrimidine bases uniquely paired, any adenine of the one molecule is always paired with a thymine of the other, and a guanine with a cytosine. There are steric reasons why these are the only pairs possible. The molecules are, therefore, precisely complementary for pairing purposes. If we designate them X and Y, then the suggestion of Watson and Crick is that X is able to act as a template for the synthesis of a second molecule of the Y type, and Y for a second X. In order to act as heterotemplates in this way the initial XY pair must previously or simultaneously separate. If this pair is previously labelled the two daughter duplices will have one labelled and one unlabelled molecular strand. This has been demonstrated in the duplication of the chromosomes (p. 136), which contain only a few strands. If RNA is templated on DNA by a similar mechanism then thymine and uracil must be interchangeable for purposes of pairing. The ribose is not involved in the pairing so that it is immaterial that the sugar differs between the two NAs.

Nearly all of the subsequent evidence has tended to support Watson and Crick's idea, notwithstanding the special difficulties at the stage of separation of the strands. Kornberg (1960) found single strands of DNA in his *E. coli* extracts and, in fact, these replicated more rapidly than the duplex strands. The single strand does not coil into a helix (Rich, 1959) so that spatial problems

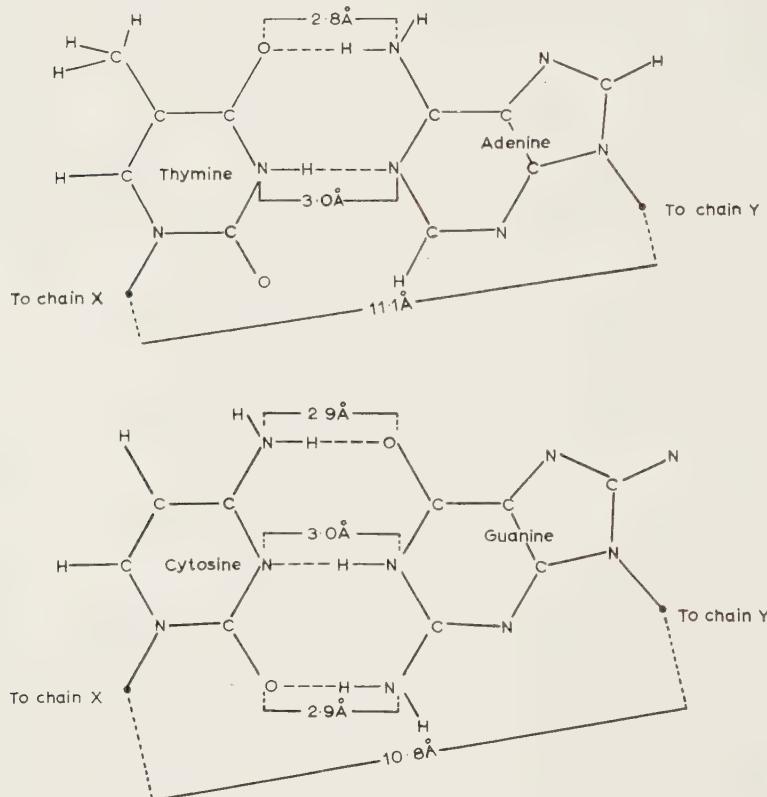


FIG. 15.3. DIAGRAM TO SHOW THE STERIC RELATIONS WHICH DETERMINE THE ONLY TWO POSSIBLE WAYS IN WHICH THE FOUR COMMON BASES OF DNA CAN BE LINKED OR "PAIRED" BY HYDROGEN BONDS OF STANDARD LENGTH

In RNA uracil replaces, and behaves like, thymine.

(Based on Watson and Crick; Pauling and Corey)

may be avoided in this way. The single strands are presumably of both X and Y types, each acting as a reciprocal template for the other, which separates as it is formed. It is not yet certain if all DNA can resolve into single strands for the purpose of replication.

Since RNA is usually single-stranded its problem of strand-separation after synthesis may be avoided in this same way. In at least some cases where it is templated on DNA this also breaks down into single strands (Leslie, 1961);

if this is general it will help to dispose of the difficulties pointed out by Zubay (1958), and avoid a more complex, encoded synthesis of RNA. However, much of the RNA of most cells is synthesized in the nucleus (Chantrenne, 1961), on the chromosomes, which have probably two strands or more per chromatid (p. 135).

In organisms with a discrete nucleus this is the sole site of DNA synthesis, though substantial amounts are subsequently passed into the cytoplasm of the oocyte, and occasionally of other cells (Zeuthen, 1951, 1952). It is the initial, if not the main, site of RNA synthesis, and there is a continuous outflow into the cytoplasm during interphase (Mercer, 1959) as well as a mass ejection at nuclear division (Mazia, 1956). Enzymes which synthesize nucleotides are among the limited number of enzymes found to be active inside the nucleus (Hogeboom and Schneider, 1952), and labelled precursors of the nucleotides pass first and mainly to the nucleus. After enucleation RNA progressively diminishes in the cytoplasm of Protozoa, whereas an isolated nucleus continues to synthesize it (Allfrey *et al.*, 1957). A "hot" nucleus containing labelled NAs, implanted in a "cold" cytoplasm passes out labelled RNA, but none of this label enters the indigenous nucleus, so that there appears to be one-way movement of RNA.

DNA synthesis occupies a brief period early in the cell cycle, while that of RNA is generally later, but more sustained. The cell increases its RNA-synthesizing machinery at the time of division so that the rate of synthesis in each daughter becomes equal to that of the parent cell (Mazia, 1961). The sudden doubling of activity, therefore, is probably due to events initiated in the nucleus.

There is reason to think that some RNA is synthesized in the cytoplasm (Leslie, 1961). This is necessarily true in bacteria, since their nuclear organization is very primitive, but in animals also, following the administration of labelled materials there is soon a rapid turnover in cytoplasmic RNA (Allfrey *et al.*, 1955a; Jeener and Szafarz, 1950; Szafarz, 1952), which probably indicates active synthesis. Enucleated halves of the alga, *Acetabularia*, continue to synthesize RNA for some time (Chantrenne, 1961). The template here is probably existing RNA.

There is no good evidence that DNA is directly convertible into RNA, but in embryos, and perhaps also under other conditions, it does appear that DNA can be formed from RNA or from its degradation products. In the young embryo RNA often seems to be the only possible source of the heavy synthesis of DNA. A similar conversion is possible in the maturing sperm (Brachet, 1957, p. 185) and in the male pronucleus after fertilization (p. 138). In *Bacillus megatherium* (Spiegelman, 1957) the enzyme RNAase destroys RNA and at the same time the ability to synthesize DNA—as well as RNA itself, and protein. This may imply that RNA is an agent for DNA synthesis, rather than a mere precursor, and such a role seems certain in bacteria infected with 'phage (p. 156); the host's RNA is used to promote the synthesis of virus

DNA, the raw materials coming from the external medium. The promotion appears to be indirect, via virus protein, and this would be consistent with the above results on *B. megatherium*, and with other indications already given in this chapter. The protein is probably that of the enzymes required for DNA synthesis.

In all, there is considerable circumstantial evidence that protein is necessary in some way for nucleic acid synthesis, though interpretation of the facts is not easy. Gale (1953, 1959a) found that in *Escherichia coli* and *Staphylococcus aureus* rapid RNA and DNA synthesis depends on the presentation of all the common amino acids, while in the regenerating nerve of the rat (Mannell and Rossiter, 1954) protein depletion causes an abnormally slow production of RNA. Adequate protein is necessary to enable RNA synthesis to benefit from any increase in energy supply (Munro and Naismith, 1953). Brachet (1957, p. 250) gives further evidence for control by protein. Certainly enzymes are required for all stages of NA synthesis, and these are probably orthodox protein enzymes.

It is possible, of course, that, in addition, amino acids directly affect NA synthesis. Glycine, aspartic acid and some other amino acids are raw materials for the synthesis of the nucleotide bases (p. 222) and this may explain why the a.a. requirement of *Bacillus megatherium* is at least partly "spared" if fully formed nucleotides are presented, though alternatively it could be due to the decreased requirement for enzymes to synthesize nucleotides. It has been found that a.a.s are still required for RNA synthesis when that of protein has been arrested by cobalt salts or by chloramphenicol (Cohen, 1959) so that a direct action of NA synthesis seems likely. In low concentration a.a.s stimulate protein synthesis and in higher concentration that of RNA also (Magasanik, 1959). This perhaps implies competition, but it would be biologically pointless to synthesize more RNA when there are insufficient a.a.s for protein synthesis itself.

On the evidence from the bacteriophage viruses (p. 153) and of other facts it is possible that protein and NA are even more intimately interdependent, each acting as a reciprocal heterotemplate for the synthesis of the other. At present there is no more direct evidence than that of Commoner (1959) from the TMV (tobacco mosaic virus), and also the fact that in some cases there is a regular alternation of synthesis of the two components. Each phase, a few minutes in duration, is just about the length of time required to synthesize the complete molecule of a protein (Loftfield, 1957). At the same time each component seems to behave as a catalyst for the synthesis of the other, small amounts continuing to be effective without further recruitment. Once active, a catalyst can promote the conversion of virtually unlimited amounts of substrate, and so there seems no call for a rigidly alternating synthesis of the two; the alternation may be concerned with control mechanisms at a higher level. A catalytic action by each seems best to account for the facts that inhibition of further synthesis of protein by cobalt salts or by chloramphenicol has no serious effect on NA synthesis and that reciprocally uranyl chloride and ionizing

radiation inhibit further NA synthesis without serious effect on that of protein. The syntheses of DNA, RNA, and protein, in fact, all have considerable independence of this kind (Chantrenne, 1961). It is, therefore, not surprising that there is often a differential rate of incorporation of  $^{35}\text{S}$ , which goes mainly into protein, compared with  $^{32}\text{P}$ , which is used mainly for NA synthesis (Holmes, 1951): this does not prove that they are completely independent in their syntheses but it indicates a less rigid gearing than the reciprocal template idea.

The synthesis of the two may be often simultaneous not merely in the cell as a whole but even in the individual molecules, as Commoner (1959) postulates for the TMV. Small molecules containing a few a.a.s and a few nucleotides have now been found in living organisms (Chantrenne, 1961, p. 116) so that this kind of simultaneity may be widespread. It would also be consistent with the close association between the a.a.- and nucleotide-activating systems (p. 197). Gale (1959a) suggests that these oligonucleopeptides may be common precursors of NA and protein. Effectively nucleoprotein is formed in a single step.

### 15.2. Nucleoproteins

This complex is found to be usually more stable than either protein or NA alone and this may be an important reason why the syntheses of the two show the mutual requirement (Greenstein and Hoyer, 1950). According to mass-action principles the formation of a stable and insoluble product should favour the further synthesis of both components, whether simultaneously or by alternating templating. A further implication is that the only significance of nucleoprotein (NP) may be for the further synthesis of its two components; indeed for RNP no other function has been clearly demonstrated. It is even possible that the synthesis of whole NP precedes that of the two free components (Chantrenne, 1952b).

Perhaps for this reason little is yet known about the actual process of conjugation between the two components (Holmes, 1951), and there is uncertainty even about the nature of the bonds between them. The combination is stoichiometric (Chargaff *et al.*, 1956), implying a true chemical union, and NP moves as a unit electrophoretically (Sevag, 1952), showing that the bonds are strong. The high proportion of the diamino a.a.s in the protein moiety of most NPs seemed to imply a simple salt linkage with the NA, but it was found that the latter retained its basophilia for alkaline dyes when in the NP state. The non-valency, electrostatic attractions of hydrogen bonds are now thought to be the effective ones (Woodhouse and Sheratt, 1952). Individually they have about the same strength as the ionic valency bonds, while in the numbers which are possible between NA and protein they equal the strength of co-valency bonds. They have the further virtue of leaving the chemically reactive groups still free for other purposes. With the same steric limitations on the particular atoms which can form the bond as those which were observed between NA molecules the combination must be strictly stoichiometric. It is suggested

that the NH<sub>2</sub> group at position 6 in adenine and cytosine may bond with the C—O group of a peptide bond, and the O— group at this position in guanine, thymine, and uracil with the NH— group of the peptide bond (Elson and Chargaff, 1957).

Inhibitors which appear to act specifically at the stage of conjugation between protein and NA have been recognized (Gale, 1953; Kopac, 1947). In neutral solution histone and NA unite spontaneously (Woodhouse and Sherratt, 1952), precipitating insoluble NP. There is also evidence that phospholipids are concerned in NP synthesis (Davidson and Leslie, 1950; Cornatzer *et al.*, 1953). It may be for this reason that P-lipids appear necessary for protein synthesis (p. 198). This need for P-lipid also strengthens other evidence that the conjugation occurs on the ergastoplasmic system (p. 146).

After its formation NP will incorporate more free a.a.s (Brunish and Luck, 1952), indicating autocatalysis of a kind. Ionizing radiations block the further uptake of <sup>14</sup>C into NP (Hevesy, 1949a), showing that the NA component is necessary for this. The uptake into other proteins is not affected. The sensitivity to irradiation in fact implies that there must be a continuous synthesis of NA, so that again a simultaneous synthesis of the two components seems probable.

### 15.3. Other Conjugated Proteins

The number of metabolites which in their active condition are conjugated with protein may be very large (J. Needham, 1942, pp. 206–12). They include many coenzymes and are usually known as prosthetic groups when bound in this way. The prosthetic group of some conjugated proteins in addition to the nucleoproteins is a nucleotide or a derivative (p. 225); in contrast to NP these have only one or two nucleotides per molecule. The co-dehydrogenases, nicotinamide-adenine dinucleotide (NAD), flavin (isoalloxazine) mono-nucleotide (FMN), and flavin-adenine dinucleotide (FAD) are rather loosely bound to their protein *apoenzymes* (Green, 1951; Racker and Krimsky, 1952), but other B-vitamin coenzymes, and the haem group of the cytochromes, are more firmly bound. Many of the conjugated proteins are chromoproteins, used for a variety of metabolic purposes since great chemical reactivity is associated with colour. Some are used in camouflage (Fox and Vevers, 1960).

In general the two moieties of these proteins are completed before conjugation, which is in contrast to the synthesis of NP. The apoenzyme combines with the completed NAD co-enzyme 2,000 times more rapidly than with free nicotinic acid (Alivisatos and Denstedt, 1952), and the globin moiety of haemoglobin is probably completed before porphyrin synthesis begins in the erythrocyte (Anon, 1953b); the iron is added after conjugation, however (p. 333). There is evidence that the PO<sub>4</sub> group and the isoalloxazine nucleus of the flavoproteins may be bonded independently to the protein before union with each other (Williams *et al.*, 1950), and so constitute an exception to the general rule, but it is known that the base can be phosphorylated first (p. 225), while during the subsequent functioning of the enzyme the coenzyme as a unit

separates from the protein, and reassociates spontaneously, in each oxidation-reduction cycle. The initial order of synthesis, therefore, should be rechecked.

The photochemical pigments of the retina resemble the flavoproteins in having this kind of physiological cycle. The cysteine residues of the protein, *opsin*, catalyse its conjugation with its prosthetic group, *retinene*, a type of autosynthesis which is particularly useful here since, once activated by light, the conjugate resolves spontaneously. Moreover, the liberated retinene is very unstable, and the opsin also is less stable than in the conjugate (Wald, 1956). Physiologically also there is a premium on rapid resynthesis. It is probably significant that cysteine residues play a part also in the conjugation of haem to protein, in cytochrome, and that they are involved in the cyclic formation and resolution of actomyosin during the muscle contraction cycle: actomyosin may be regarded as a conjugate protein.

Organisms unable to synthesize the prosthetic groups, haemin and pyridoxine may nevertheless continue to synthesize the apoenzyme with which these usually combine (Cohen, 1959). At the same time either the synthesis is depressed by as much as 90 per cent or the unconjugated protein is much more unstable than the normal holoenzyme. If the prosthetic groups are added to these systems they rapidly conjugate with the apoenzyme (Chantrenne, 1961).

Glycoproteins and lipoproteins resemble the nucleoproteins in having a higher proportion of the non-protein moiety than most other conjugated proteins. Some aspects of their synthesis have been considered in connexion with skeletal materials (p. 166) and other aspects will be considered in the next section. The lipid is so firmly bound to the protein that proteolytic enzymes do not remove it or reveal its lipid properties (Lovern, 1949). An outstanding feature of the glycoproteins is the variety of bonds between the two components, in contrast to the monotony of the glycosidic link within the polysaccharide moiety and of the peptide link within the protein (Gottschalk *et al.*, 1962).

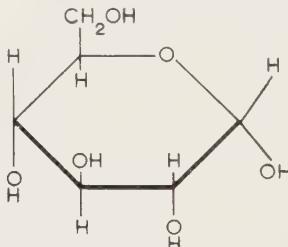
#### **15.4. Other Substances Synthesized by Animals**

The number of other major syntheses in animals is limited. Some of the materials on which they feed, the vitamins and many of the lipids, including some of the neutral fats, are absorbed and utilized without significant change. Those fats with short-chain fatty acids are hydrolysed for absorption from the gut and immediately resynthesized. The high polymers, proteins, nucleic acids, and polysaccharides are resolved to monomers in the gut and reassembled in host specific patterns, as already described. The repolymerization of monose carbohydrates, and of their derivatives, to polysaccharide is relatively simple, monotonous, and taxonomically non-specific, and rather significantly it does not require the aid of nucleic acids (Mercer, 1958). Much, but by no means all, of the polysaccharide and lipid is simply a fuel store and not part of the living fabric, and so for a number of reasons there are few other syntheses which merit detailed consideration here. Details of the basic pathways of biosynthesis

by autotrophes may be obtained from the standard textbooks. Many of these pathways, including those of the B-vitamins, as well as other more complex molecules, are described by Kit (1960). Here it will be adequate to indicate briefly the scope of biosynthesis in heterotrophes, in so far as it is necessary for the general understanding of growth at this level, and where it illustrates general principles of biosynthesis.

#### 15.4.1. Carbohydrates

The biosyntheses of polysaccharides and fats are interesting also for comparison with their pathways of catabolism, which are perhaps more directly relevant to growth processes than their syntheses (p. 258). The most common monose resulting from carbohydrate digestion is  $\alpha$ -D-glucose—



It can be used as a fuel directly, after phosphorylation to  $\alpha$ -glucose-6-P by ATP; alternatively this is isomerized to  $\alpha$ -glucose-1-P and polymerized to glycogen (glycogenesis) by a series of enzymes. The process is sometimes called transglycosidation, because whole polymers, as well as single glucose residues can be conjugated in the same way; it is an interesting parallel to transpeptidation (p. 194). One of the enzymes, the branching factor, can transfer a long polymer chain from one part of the polysaccharide molecule to form a branch elsewhere, several of the alcohol groups of the monose residue being capable of the ether type of condensation. As a result of transglycosidation there may be a geometric mode of increase (p. 203), as well as the more usual linear extension of the amylose chains. The high energy available in the  $\sim$ P bond of the glucose  $\sim$ P is retained in the amylose linkages and so the branch-transfer also requires no additional energy supply. When glycogen is broken down for use inorganic P can be used for the initial phosphorylation of the glucose residues, for this same reason; the resulting glucose-1-P still has the energy-rich bond.

Also mainly for this reason it was supposed that the pathway of glycogen synthesis was a simple reversal of the pathway of catabolism, particularly since this uses a phosphorylase as its main enzyme inside cells, in contrast to the simple hydrolases used in intestinal digestion. However, it now seems (Krebs, 1962) that, as in the case of proteins and nucleic bases, and perhaps quite generally, the two pathways are different. For glycogen synthesis uridine triphosphate (UTP) is a cofactor, and the energy of a second  $\sim$ P bond is

expended to link the glucose-1-P residues, so that the process is much more rapid and spontaneous than synthesis by phosphorylases; under selected experimental conditions *in vitro* the latter does, of course, work quite effectively in the direction of synthesis. The use of UTP in glycogen synthesis possibly has some significant resemblance to the use of cytosine triphosphate in phosphatide synthesis (p. 239); it seems probable that the nucleotide triphosphates are used very widely in biosynthesis, quite apart from the generic use of ATP as a source of energy and the use of NAs specifically for the synthesis of peptides.

The other two common monoses resulting from the digestion of food carbohydrates, namely galactose and fructose, also are polymerized to glycogen, after conversion of their phosphates to glucose phosphate. Galactose gives initially glucose-1-phosphate and fructose gives glucose-6-phosphate. Some molluscs synthesize galactogen instead of glycogen, and have predominately galactose instead of glucose in their mucus (p. 246). Galactose also occurs fairly widely in some other materials synthesized by animals. The locust, *Schistocerca*, converts the glucose absorbed from the gut into the insoluble disaccharide, trehalose, as an aid to rapid absorption (Treherne, 1958). Some elasmobranchs store large quantities of scyllitol, one isomer of the hexahydric alcohol, inositol (p. 296), probably as fuel (Fischer, 1944); it may, therefore, be regarded as an alternative to a carbohydrate store. Ruminants are capable of building up the C<sub>3</sub> fatty acid, propionic acid, into sugar, while various other C<sub>3</sub> molecules such as glycerol, as well as acetic acid, also can be used in this way by animals. It is still generally believed that the pathway of synthesis from these precursors is the reverse of the glycolytic sequence, except at one controlling step (Baldwin, 1953, p. 405), but in view of recent discoveries about the glucose-to-glycogen pathway the difference may prove to be greater. This is relevant also to the resynthesis of glycogen from the lactic acid produced in anaerobic respiration.

Mammals (Hall *et al.*, 1958) and tunicates are able to convert  $\alpha$ -glucose to  $\beta$ -glucose in the process of polymerizing it to animal cellulose (p. 166) while arthropods and other groups can aminate and acetylate the  $\alpha$ -glucose and then polymerize it to the  $\beta$ -polymer, chitin. There is an enzyme which inverts the bond to the  $\beta$ -form (Florkin, 1960, p. 263). The vertebrates conjugate acetyl-glucosamine with glucuronic and sulphuric acids and polymerize this unit to form the mucitin sulphate of mucus and the connective tissue (Kent and Whitehouse, 1955). Chondroitin sulphate of the ground substance of cartilage, etc., is an analogue of mucitin sulphate, with galactose replacing glucose; here the sulphation step is known to be the last in the pathway of synthesis (Whitehouse and Lash, 1961), and ATP acts as transferase for the radical.

Pentoses occur not only in nucleic acids but also in the antigens of the ABO blood group as well as in human milk, in the egg-jelly of echinoderms, and elsewhere. In animals they are formed by degrading glucose, in the gluconic acid pathway (p. 259) and not by progressive synthesis as in autotrophes.

Animals are capable of a number of fundamental syntheses in this general

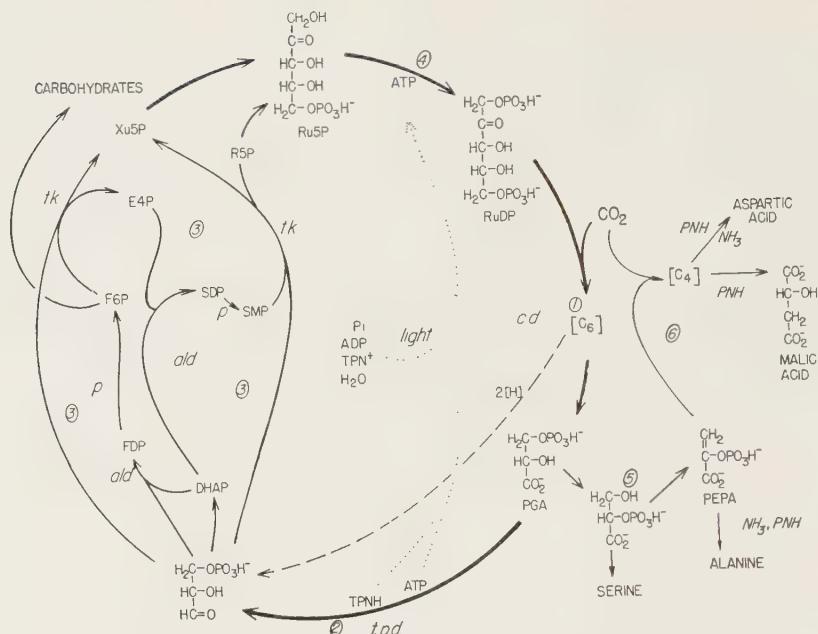


FIG. 15.4. CARBON FIXATION AND REDUCTION IN PLANTS

(1) Ribulose diphosphate reacts with  $\text{CO}_2$  to give an unstable 6-carbon compound which splits to give two 3-carbon compounds, one of which is 3-phosphoglyceric acid (PGA). The other 3-carbon compounds might be either 3-PGA, as it is known to be in the isolated enzyme system, or some other 3-carbon compound such as a triose phosphate (dashed arrow). (2) PGA is reduced to triose phosphate with ATP and TPNH derived from the light reaction, and water. (3) Various condensations and rearrangements convert the triose phosphate to pentose phosphates. (4) Pentose phosphate is phosphorylated with ATP to give ribulose diphosphate. Further carbon fixation occurs via conversion of PGA to phosphoenolpyruvic acid (5), and carboxylation (6), to form a 4-carbon compound (probably oxaloacetic acid). Reactions leading to the formation of some of the secondary intermediaries in carbon reduction are also shown. *ald.*, aldolase; *c.d.*, carboxydismutase; DHAP, dihydroxyacetone phosphate; E4P, erythrose-4-phosphate; F6P, fructose-6-phosphate; FDP, fructose diphosphate; PEPA, phosphoenolpyruvic acid; PNH, reduced nicotine-adenine dinucleotide; R5P, ribose-5-phosphate; Ru5P, ribulose-5-phosphate; RuDP, ribulose diphosphate; SDP, sedoheptulose diphosphate; SMP, sedoheptulose monophosphate; *t.k.*, transketolase; *t.p.d.*, triose phosphate dehydrogenase; TPN, TPNH, oxidized and reduced forms of nicotine-adenine dinucleotide phosphate; Xu5P, xylulose-5-phosphate.

(From M. Calvin, 1962)

field, however, that is to say among water-soluble substances containing only the elements C, H, and O. They can even "fix"  $\text{CO}_2$ , mainly by an effective reversal of particular decarboxylation reactions of the Krebs cycle (Krebs, 1951; Florkin, 1960). Decarboxylation is used to yield energy, and the reverse reaction, therefore, must be primed by ATP. The fixation of  $\text{CO}_2$  differs

from that by green plants (Fig. 15.4) in using stored chemical energy instead of photic energy to synthesize the ATP, from ADP. There is also a difference in site of fixation since plants fix CO<sub>2</sub> into a carbohydrate, via a pathway with considerable resemblance to the gluconic acid pathway, operating in reverse.

There is considerable evidence that animals can fix CO<sub>2</sub> on to acetate and so build carbohydrate from fatty acids. Both labelled acetate and labelled CO<sub>2</sub> can be administered and the label recovered from glucose. This synthesis is best seen in ruminants, which produce large amounts of acetate from the bacterial fermentation of cellulose in the rumen. This fixation again is effectively (but not necessarily directly) a reversal of a common decarboxylation step of respiratory metabolism, that of pyruvate.

In principle, of course, it is uneconomic to oxidize and decarboxylate material and then to attempt to use the energy for the refixation of CO<sub>2</sub>, in order to synthesize particular hydroxy acids or their derivatives. It should be possible, in principle, to tap off intermediaries of the Krebs cycle at any point, whichever way the cycle is turning. However, it is probable that the cycle runs an express service in the catabolic direction and that, in consequence, synthesis must proceed by a local, stopping service on the up-line.

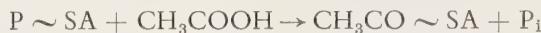
Certainly these local stations and their branch lines are metabolically important, quite apart from the respiratory function of the cycle. The most important in connexion with growth are those leading to the amino acids, glutamic and aspartic acids, synthesized by the amination of  $\alpha$ -ketoglutaric acid ( $\alpha$ -KG) and oxaloacetic acid (OAA) respectively, both members of the Krebs' cycle. In the same way pyruvic acid is aminated to alanine (p. 242). Glutamic acid (p. 289) is the key member because free ammonium ion can be used to aminate  $\alpha$ -KG, whereas the others are formed by transamination from glutamic to their  $\alpha$ -keto precursors. All three can transaminate to the  $\alpha$ -keto precursors of other a.a.s.

When the Krebs cycle is working in the direction of synthesis, presumably it cannot be used so effectively for respiratory purposes and there is actual evidence (Roberts, 1959) that glycolysis is then used more extensively as an alternative. This may help to explain the predominance of glycolytic respiration in rapidly growing tissues (p. 259). If acetate alone is available as a source of energy, however, it must be oxidized via the Krebs cycle and this then ceases to operate in the direction of synthesis. This response implies that there must be a direct reversal of some steps at least of the Krebs cycle, when it operates in synthesis, so that it is necessary to keep an open mind on the general question of irreversibility. The indication from the present section is that all three respiratory paths, glycolytic, gluconic, and Krebs, may be more fully reversible than most pathways.

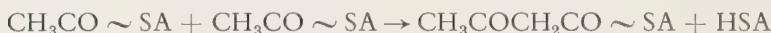
#### 15.4.2. Lipids

As already indicated the glyceride lipids are absorbed from the gut either unchanged or after simple hydrolysis, and much of the body glyceride changes

quite passively with the nature of the dietary fat. Ruminant mammals, with the aid of their rumen flora are able to convert much of their dietary cellulose via acetic and butyric acids to fats of the body and milk. Acetate is, therefore, a key metabolite for the syntheses and interconversions of carbohydrates and fats. Animals in general convert carbohydrate to fat by glycolysis to pyruvic acid and decarboxylation to acetic; various fatty acids are then synthesized from the acetic residues. In anaerobic organisms this may be effected by a reversal of the familiar catabolic path of  $\beta$ -oxidation (Baldwin, 1953; Green, 1960). The necessary energy for conjugating the acetic residues may be provided by transacetylase (p. 313) which, with the help of ATP, activates its substrate by forming an energy-rich bond with the SH-group of the enzyme—



where HSA is the enzyme. Two active acetate groups may then be conjugated with the release of one molecule of the enzyme—



After reduction in the  $\beta$ -position the resulting and still "active" butyrate may condense with a further active acetate and so on. Most of the natural fatty acids have an even number of C atoms in their molecule,  $\text{CH}_3 \cdot (\text{CH}_2)_n \cdot \text{COOH}$ , showing that synthesis is effectively by the condensation of  $\text{C}_2$  units. However, the oxidation of fatty acids as a source of energy requires both transacetylase and ATP, and active acetate units are liberated, so that some additional factor presumably would be necessary to make the process operate in reverse.

It is, therefore, important that Green (1960) has now shown that in aerobic organisms fatty acids are not synthesized by a simple reversal of their pathway of oxidation. The initial acetate residue is activated in the same way, by conjugation with CoA but it then condenses with  $\text{CO}_2$  and not with another acetate. Moreover, the resulting intermediary compound is not pyruvate but malonate. This itself is a powerful poison because it is competitive analogue of succinic acid, a member of the Krebs cycle; in fatty acid synthesis, however, malonate presumably is harmless because it is in a conjugated form. The  $\text{CO}_2$  also reacts as an active form, conjugated with the B-vitamin, biotin (p. 310). When it combines with acetyl CoA the resulting malonate remains conjugated with CoA. It then reacts with a further acetyl CoA unit to form a  $\text{C}_5$  compound. This is reduced and decarboxylated to butyryl-CoA, which reacts with a further molecule of malonyl-CoA to form a  $\text{C}_7$  compound, and so on. As in many other pathways the steps seem very numerous and circuitous. Notwithstanding this, the pathway of fatty acid synthesis in effect involves the progressive addition of  $\text{C}_2$  units, as so much circumstantial evidence has always indicated.

On the other hand the C<sub>5</sub> intermediary helps to explain the series of lipids synthesized from isoprene (see below).

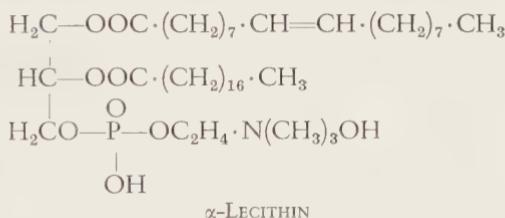
Manganese is a cofactor for the synthesis, in contrast to Mg<sup>++</sup> for the β-oxidation, of fatty acids. Reduction is by a dehydrogenase containing NADP (p. 308) as coenzyme, whereas oxidation of fatty acids requires the NAD coenzyme. NADP is more generally concerned in biosyntheses than NAD.

The formation of triglycerides from fatty acids again is not a simple reversal of the reactions of hydrolysis, such as those which occur in the gut, although the equilibrium for some lipases lies as much as 60 per cent in favour of esterification (Baldwin, 1953, p. 106), and even more at an acid pH (Scott, 1953). Both components appear to need special activation (Kit, 1960; Florkin, 1960). The active form of glycerol is the α-phosphate—



which as a point of incidental interest becomes optically active in the process of phosphorylation, since the two terminal C-atoms are now different. The fatty acids are activated by conjugation with coenzyme A and so probably continue directly from their synthesis to the esterification. When fatty acids have been conjugated in turn at two positions, to give a diacylphosphatidic acid, the third displaces the phosphoric acid at the α-position and the tri-glyceride is complete.

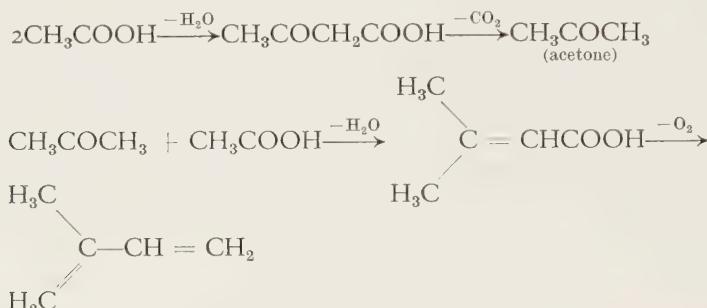
Phosphatides such as lecithin and cephalin are synthesized by a similar pathway except that phosphorylcholine or one of the other phosphorylated



bases is substituted at the α-position. For this purpose the P-base first reacts with cytidine triphosphate (CTP) to form a dinucleotide analogue, cytidine diphosphoryl choline, etc.—a further use of nucleoside polyphosphates in biosynthesis. The bases choline and ethanolamine are synthesized by animals in a common pathway from the a.a. serine (Kit, 1960), the methyl groups for choline being supplied from methionine (p. 289).

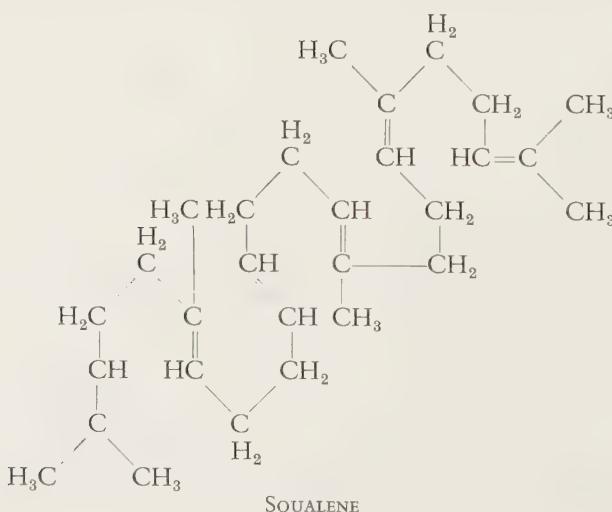
Vertebrates, but not all animals, are able to synthesize cholesterol, which appears to be the parent steroid, the whole way from acetate (Popjak and Cornforth, 1960). The intermediate, somewhat unexpectedly, is a C<sub>5</sub> unit, —CH<sub>2</sub>·C(CH<sub>3</sub>)—CH·CH<sub>2</sub>—, which is related to isoprene, and is also the basis of rubber, the terpenes and many other essential oils, and other plant products,

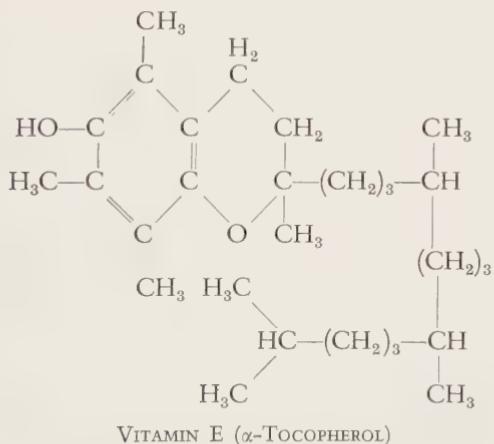
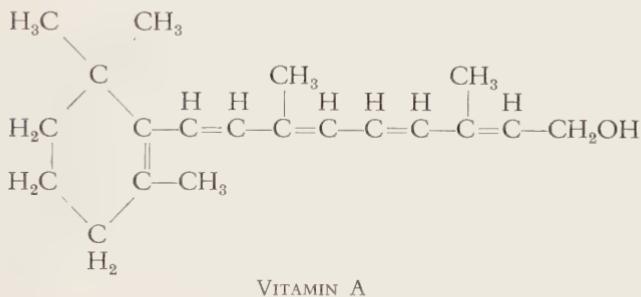
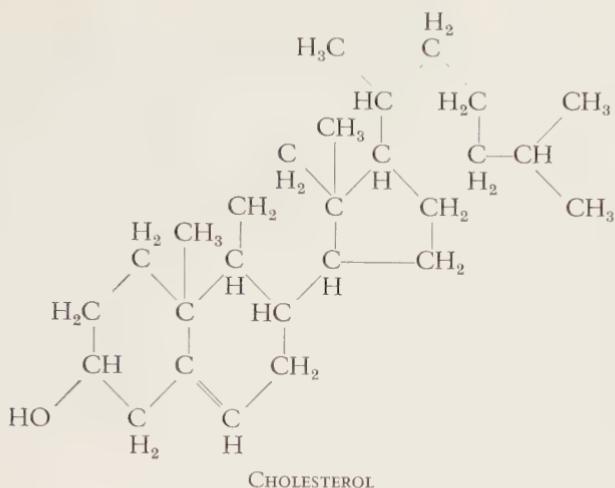
including vitamins A, E, and K and the carotenoids. Most are simple polymers of the C<sub>5</sub> unit, which is synthesized as follows—



Six of these C<sub>5</sub> units form the open-chain hydrocarbon, squalene, which occurs in elasmobranchs, and this undergoes ring closure, with the loss of three carbon atoms. The process takes place in the supernatant and on the ergastoplasm, like protein synthesis. From cholesterol the vertebrates form other steroids, in particular the gonadal and adrenal-cortical hormones, the bile acids and vitamin D. The latter is interesting in being formed from its intermediary, ergosterol, in the mammalian skin, under the influence of ultraviolet rays.

Vitamin A is structurally a tetramer of isoprene, and the structures of the other fat-soluble vitamins, E and K, imply the same origin, by the same characteristic pattern of CH<sub>3</sub> side-chains and double-bonded pairs of C atoms. Nevertheless, mammals appear unable to synthesize these substances directly, one of the many mysteries of biosynthesis. The explanation may be the same as for B-vitamins (p. 303), that there is a guaranteed supply in the natural diet, and the mechanism of synthesis, therefore, has been economized (Lwoff, 1949).

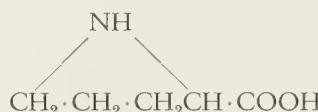




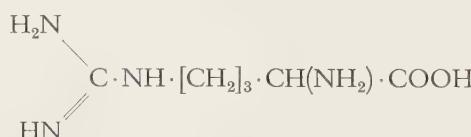
#### 15.4.3. Amino Acids and Other Nitrogen Compounds

As already indicated (p. 183) animals themselves synthesize some of the biological amino acids, which, therefore, are said to be non-essential,

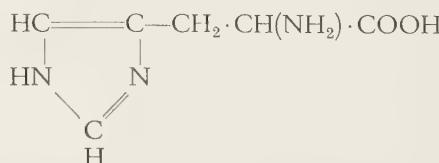
whereas the others are essential dietary constituents. The key role of glutamic acid in synthesizing the former group has been mentioned, and also the way in which it and aspartic acid, HOOC·CH<sub>2</sub>·CH(NH<sub>2</sub>)·COOH, and alanine, CH<sub>3</sub>·CH(NH<sub>2</sub>)·COOH, are formed by amination of their  $\alpha$ -keto precursors. Alanine is the main source of serine, HOCH<sub>2</sub>·CH(NH<sub>2</sub>)·COOH, and the latter of glycine CH<sub>2</sub>(NH<sub>2</sub>)·COOH, which is structurally the simplest of the a.a.s. Serine also provides the skeleton for cysteine, HSCH<sub>2</sub>·CH(NH<sub>2</sub>)·COOH, the —SH group coming from the other S-containing a.a., methionine (p. 289). Those organisms which can synthesize methionine start from aspartic acid, which is the main source also of threonine, CH<sub>3</sub>·CHOH·CH(NH<sub>2</sub>)·COOH; both methionine and threonine are dietary essentials for most animals, however. They are able to synthesize proline—



which is unique among the common biological a.a.s in forming a closed ring through its  $\alpha$ -NH<sub>2</sub> group; glutamic acid is the precursor. The branched-chain a.a.s, valine, (CH<sub>3</sub>)<sub>2</sub>·CH·CH(NH<sub>2</sub>)·COOH, and its C<sub>6</sub> relatives, leucine and isoleucine, are dietary demands for most animals investigated; autotrophes synthesize them from other a.a.s and from pyruvic acid, and not via isoprene (p. 239) as might have been anticipated. Another essential a.a., lysine, H<sub>2</sub>NCH<sub>2</sub>·[CH<sub>2</sub>]<sub>3</sub>·CH(NH<sub>2</sub>)·COOH, is synthesized by two different pathways in microorganisms. Arginine—



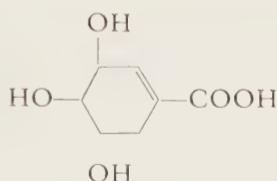
is synthesized from ornithine, H<sub>2</sub>N[CH<sub>2</sub>]<sub>3</sub>·CH(NH<sub>2</sub>)·COOH, in the course of urea formation, in many animals, but this is not available as arginine for their body's proteins, and this a.a. also is a dietary demand. Microorganisms synthesize it via ornithine and citrulline, from glutamic acid, the section of the path from ornithine onwards being the same as in the Krebs-Henseleit urea cycle. Histidine—



is a dietary essential.

In autotrophes it is probably synthesized from a pentose sugar, the nitrogen groups coming from more than one source (Kit, 1960).

The three aromatic a.a.s of the body's proteins, phenylalanine, tyrosine, and tryptophan, are synthesized by autotrophes mainly from glucose, via shikimic acid—



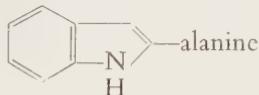
Insects, among heterotrophes, can perform this synthesis (Brunet, 1963), but perhaps only through their microorganisms. Some of the intermediaries are still unknown but a pyruvate side-chain is added, to form prephenic acid, the precursor of phenylalanine—



and of tyrosine—



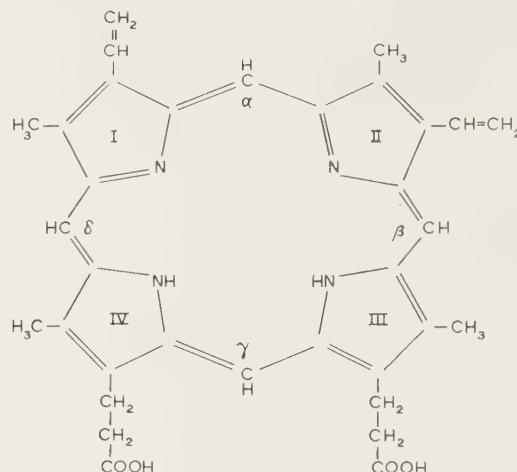
Anthranilic acid is the precursor of the indole nucleus of tryptophan—



while serine is the source of its alanine side-chain.

Although nitrogen compounds in general are the most complex, and include many which are demanded as dietary constituents by animals, it is among these that their most radical and impressive syntheses are effected. In addition to the nucleotides (p. 221) and the phosphatides, there is the synthesis of the porphyrins (Lemberg and Legge, 1949; Shemin and Wittenberg, 1951), the prosthetic groups of the haemo- and myoglobins, and of the cytochromes and other enzymes. This is among the most important and certainly the most impressive of animal biosyntheses. The raw materials are glycine and acetate, the latter being converted to succinic acid or a related metabolite via the Krebs cycle. This unites with glycine and two molecules of the resulting intermediary then unite to form porphobilinogen, consisting of one pyrrole ring bearing side-chains. One of these is used to join the unit to another, a chain of two pairs of them then closing to form a second-order ring, the porphin nucleus. Proto-porphyrin, the most common biological derivative, has the structure shown below, the side-chains being in place before condensation of the pyrrole units, as already indicated. In view of these fundamental and extensive syntheses it

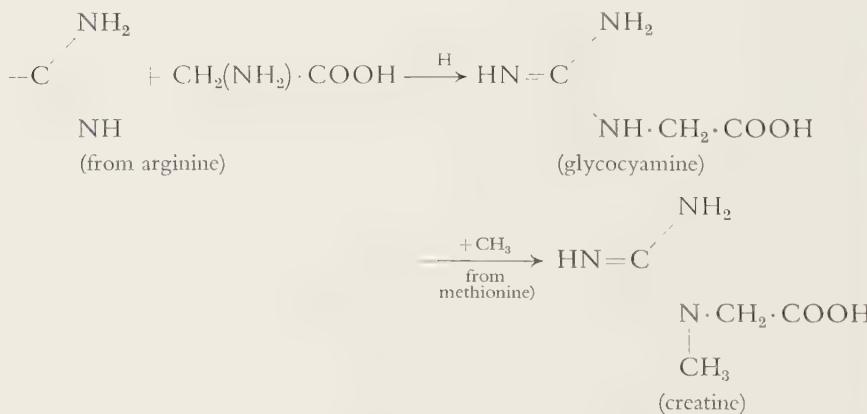
seems probable that the inability of animals to synthesize the B-vitamins is purely opportunist, as already suggested for the fat-soluble vitamins.



#### PROTOPORPHYRIN

According to convention a carbon atom is assumed to be at every ring position not specified.

The vertebrate phosphagen, creatine phosphate, a reserve of  $\sim\text{P}$  for the regeneration of ATP from ADP, is itself synthesized from glycine—by “transamidination” from arginine, followed by methylation—



Like the precursors of the purines it is phosphorylated before completion, at the glycocyamine stage.

Other materials synthesized by animals include a number of pigments, many of them with heterocyclic nuclei and synthesized by extensively oxidative pathways. The carotenoid chromophor, retinene (p. 233), therefore, is rather exceptional in being non-nitrogenous, in having a long hydrocarbon chain

(p. 241) and in being obtained in almost completed form in the diet (p. 299). It is also one of the few groups of fat-soluble pigments (Fox and Vevers, 1960). Melanin is an indole complex but is synthesized from tyrosine or other phenolic precursors (Mason, 1955). Nevertheless there are pigments formed from the indolic a.a., tryptophan, namely the ommochromes and the indigo pigments. The purines and porphyrins, already considered, include a number of pigments; most porphyrins in fact are brightly coloured. The pterin pigments of animals also are synthesized, or at least completed, *in situ* (Ziegler-Günder, 1956). The pathway does not pass via pyrimidines or purines (p. 222), as might have been expected from their structural relationships, but this is no longer a cause for surprise perhaps (p. 320). Moreover some animals, at least, demand the pterin derivative, folic acid (p. 314), ready-made, in their diet, and reciprocally, folic acid will not serve as a source of the pterin pigments. In spite of the independencies in these pathways, others which have no structural relationship are sometimes linked, for instance the ommochrome with the melanin pathway (Butenandt *et al.*, 1956). Pigments are often topographically associated for functional reasons, either in camouflage, or in metabolism, or both, and this may complicate the synthesis mechanisms also.

Any complete survey of animal syntheses should also consider the many other important metabolites formed from phenylalanine and tyrosine as parent substances. These include thyroxin (p. 373), adrenalin, tyramine, and the tanning phenols (p. 166). Animals also synthesize a considerable number of metabolites from the other amino acids, collectively, and these are described in the standard biochemistry textbooks. They must be taken into consideration when assessing the importance of the a.a.s as agents for growth (p. 288).

#### 15.4.4. Phosphorus and Sulphur Compounds

A number of these have been considered already in other contexts. Phosphorus always occurs as a substituted orthophosphate or pyrophosphate, acting either as phosphate anion or as phosphoryl radical, depending on the point of rupture in the —OH<sub>n</sub> combining groups. Hydrolysis of most of the esters yields approximately 3,000 calories per gramme equivalent, while transfer of the phosphoryl radical from anhydride to other linkages usually yields much more (Burton, 1958), if not as much as the 12,000 calories per gramme equivalent initially calculated. This is the group transfer potential (Cohen, 1959), and not the total energy of breaking the bond; this latter, in fact, is endothermic to the extent of 50 to 100 kilocalories. The bond, therefore, has a high group transfer potential, but it is popularly said to be "energy-rich," and is represented by ~P. It includes the links of P with the >C=O group of glyceric and acetic acids, the enol group of pyruvic acid and of some phenolic compounds, the —NH group of the phosphagens and other phosphate groups, in the pyrophosphates.

Many compounds of both types occur in living organisms. In the phosphoproteins there is an ester link (p. 196), and esters are formed with many

carbohydrates and alcohols, such as vitamin E (p. 299). The phosphatides are glycerol esters, and the nucleotides also have only this energy-poor bond. Hence only their pyrophosphates are very active metabolically, but these have outstanding importance. The key role of ATP as the universal mediator both of high energy and of P has already been manifest. Given a supply of respiratory energy for regeneration of ATP no phosphorylation process appears to present difficulty in the body. The formation of phosphate skeletons has been considered (p. 177).

Sulphur occurs in three main forms in the fabric of animals: (1) as the SH— group in the amino acid cysteine (p. 242), in its derivative glutathione (p. 287), and in the mercaptoethanolamine of pantetheine (p. 313), (2) as sulphur bonded between two carbon atoms, or between C and N, as in the amino acid methionine (p. 289), and the vitamins thiamine and biotin, and (3) as sulphate, in chondroitin and mucoitin sulphuric acids and other compounds (Roy, 1960). Free  $H_2SO_4$  is found in some tunicate blood corpuscles and elsewhere. Up to 2 per cent of proteins may be sulphur, and in plants the amount increases with age (Davenport, 1899).

As already noted (p. 242), dietary methionine is demanded by most heterotrophes but they are able to use its S to form cysteine from serine. The S-containing vitamins are demanded ready-made. Animals do not assimilate elemental sulphur very extensively (Davenport, 1899), or  $H_2S$  (Hevesy, 1948), but they can utilize sulphate (Dziewiatkowski, 1953) and sulphite (Kit, 1960) and reduce them to the —SH stage. Somewhat surprisingly, perhaps,  $-SO_4$  is not very extensively absorbed from the gut, although sulphur is used in that form for a number of materials. Organic sulphur, and especially sulphydryl, is very readily taken up by the tissues and this is one of the reasons for the danger of mustard gas; it forms very stable compounds with fabric materials.

### 15.5. Conclusion

The first generalization concerning biosynthesis is that the pathway is rarely a simple reversal of the pathway of catabolism of the same substance. Since the synthesis is usually strongly endergonic the precursors must be activated, usually by ATP ultimately, and often via the other nucleotide triphosphates. This is true even for the polynucleotides, or nucleic acids, themselves. Another important generalization is that structurally related substances do not necessarily share a common pathway of synthesis. This may be because it is necessary to control their syntheses independently. The limitations in the biosynthetic powers of animals appear to be opportunist, since they are capable of some very fundamental and difficult syntheses when there is no other way of acquiring indispensable metabolites. The total number of biosyntheses performed by animals is very impressive.

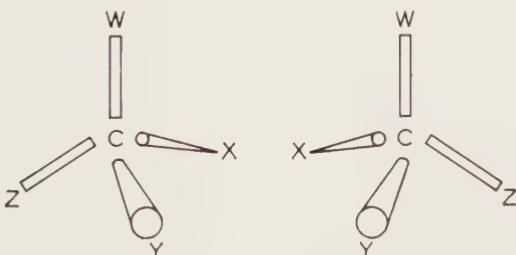
#### 15.5.1. The Specificity of Biosynthesis

Although the number of organic substances synthesized by living organisms is impressive enough it is still a very small fraction of the total number theoretic-

ally possible. Biosynthesis, in fact, has exploited a limited but highly specific selection of substances with uniquely useful properties. The "final," or biological, cause of this concerns primarily the students of evolution and genetics, and it is the "efficient," or physiological, basis which is the real concern of the auxologist. This is an important aspect of the control of growth, so important that there is a tendency to take it for granted, and at present little can be said about it except that it must have a genetic basis (Chapter 24), just as much as the more parochial, taxonomic specifications.

For the present purpose, of narration, we may recognize as the first grade of specificity, that only certain classes of material are extensively synthesized: amino acids and proteins, nucleotides and nucleic acids, sugars and polysaccharides, glycerides, phosphatides, steroids, and the B-vitamins. The second grade is that within each of these classes only a few members are widely exploited and in some cases, particularly among the B-vitamins, there is only one biologically significant member of a class. It is now a commonplace that isomers of biologically important molecules are never adequate substitutes for them, physiologically. Often they are positively deleterious, like a key which almost fits the lock, and it no longer causes surprise that normally they are not synthesized in appreciable quantity by living organisms.

There is one special case, however, a third grade of specificity, where this fact does still excite wonder; it is the case of optical isomers. These are molecules which are identical except for the way the four atoms or radicals with which it is bonded are arranged around a particular carbon atom. The situation does not arise if two or more of these radicals are alike, since there is then only one arrangement, bearing in mind that the four bonds of the carbon atom are directed towards the corners of an imaginary tetrahedron, and that the molecule in solution is free to rotate in all directions. There are two arrangements, however, when all four groups are unlike, and the carbon atom is said to be *asymmetric*; the two are mirror images of each other—



Their optical property is that a solution of the one isomer or antipode rotates a beam of plane-polarized light clockwise (dextro-rotatory) and the other anti-clockwise (laevo-rotatory). In a racemic mixture of equal parts of the two the net rotation is zero so that the degree of rotation, knowing that of the pure antipode, can be used to measure the degree of optical purity of a preparation.

The difference between the two is essentially physical, and so *a priori* there seems no reason why they should be found to differ in chemical properties, or why living organisms should discriminate between them. It was therefore surprising to find that the dextro- and laevo-rotatory forms, in fact, often have significantly different properties, and are sometimes entirely different physiologically. For instance L-tryptophan and L-asparagine are sweet and the D-forms tasteless, while the biological antipodes of adrenaline and nicotine have powerful neurohumoral actions and the "unnatural" forms are almost inactive. Once these differences are recognized there is no more cause for surprise, on purely biological grounds, that living organisms synthesize and exploit only the one optical isomer, than that they have exploited only a particular twenty of the possible  $\alpha$ -amino acids (p. 183). On biochemical grounds, however, it is much more remarkable. At the same time it is a problem particularly suitable for experimental attack because it deals with a simple alternative. Through it the general problem of uniqueness in biological synthesis seems specially exposed to research, for in the chemical laboratory a racemic mixture of the two antipodes is usually synthesized. There has been much stimulating speculation about the nature of the mechanism in living organisms and its evolutionary origin (Mills, 1932; Gause, 1941; Richie, 1948; Kuhn, 1958).

In fact the two antipodes are rarely produced in exactly equal amounts even in the chemical laboratory, the one preponderating by as much as 0·21 per cent (Mills, l.c.) which is probably enough to have been further exploited by living organisms. Moreover, when a racemate of a substance A is allowed to combine with one of B, then the ratio of the amount of compound  $A_L \cdot B_L$  to that of  $A_R \cdot B_R$  may be greater than both  $A_L/A_R$  and  $B_L/B_R$  (Langenbeck and Triem, 1936). Even some inorganic catalysts favour the one antipode, often by as much as a factor of 2, and in a cyclic series of reactions such as those which are typical of metabolism there could be a spontaneous, progressive enrichment.

Although the racemate is the condition of maximal entropy, and racemization is spontaneous, Langenbeck (1935) concluded that a system of racemic compounds would be thermodynamically unstable and that any disproportion between the antipodes would tend to increase. Frank (1953) came to much the same conclusion from a consideration of the kinetic equations of growth in self-reproducing systems of the type found in living organisms. Much earlier Karl Pearson had already concluded, from geometrical considerations, that a disproportion between the two antipodes must appear spontaneously at a certain level of molecular size (Pirie, 1957). This is not restricted to compounds having asymmetric carbon atoms, or even to carbon compounds in general; quartz crystals occur in antipodal forms and it is among large crystals that a clear preponderance of right-handed over left-handed forms is found (Pirie, 1952). It therefore seems that there is nothing very peculiar in the tendency towards a high preponderance of one antipode in living systems. Not only does synthesis tend to be biased but there can be a spontaneous deracemization

after formation; there is normally some degree of continuous mutual interconversion of antipodes, which is the basis for the more common racemization tendency, but if one antipode is more stable than the other, for any reason, then the interconversion is biased and all may become trapped in the stable form. Such substances are said to be *stereoautonomic*. Again, some racemic mixtures spontaneously "resolve" into their antipodes and it was this which enabled Pasteur to make his classical laboratory resolution of the tartaric acids: pure crystals of the two antipodes form and he separated them by hand, under the microscope.

Kuhn (1958) believes that the high degree of optical purity of virtually all biological asymmetric compounds may depend on a limited number of stereoautonomic substances acting as "pillar" substances, or optical templates, for the rest and, in fact, there is considerable evidence that asymmetry begets asymmetry. Proteins selectively absorb one antipode of certain other substances from a racemic mixture (Bradley and Brindley, 1954), probably depending on their own net asymmetry. Similarly the ability of enzymes to catalyse highly preponderant synthesis probably depends on the optical purity of their constituent amino acids and prosthetic groups; the synthesis of one antipode may be as much as 1,000 times as rapid as the other, compared with a maximal preponderance of twofold for simple catalysts. By contrast the amino acids synthesized from simple symmetrical molecules by electrical discharge are racemic mixtures (Florkin, 1960).

The idea of optical templates and interdependent preponderance is supported by the fact that all the biological sugars are D-forms, that is to say the asymmetric carbon atom farthest from the CHO (or C:O) end of the molecule has the same asymmetry relations in all. They are said to be all D-forms because the simplest member, glycerose, is dextro-rotary, but the longer-chain members have several asymmetric carbon atoms and their net rotation is the algebraic sum of the actions of all these: it may be dextro or laevo. The biological amino acids are probably all L-forms, structurally derivable (but not necessarily actually derived) from L-glycerose. The implication is that there may be one optical template for the sugars and one for the amino acids. For the reason already given the actual rotation of some natural a.a.s is dextro and this complication may obscure optical family relationships among more complex biological molecules.

It is not universally accepted that biological optical purity depends on a few stereoautonomic compounds, since it is clear that some degree of natural preponderance is almost universal. As the basis of this various natural asymmetries have been suspected: the direction of rotation of the earth, the asymmetry of polarization of sunlight by air and water, the discrepancy between geographical and magnetic poles, and so on, perhaps even the fundamental non-parity property of the physicist (Bayliss, 1956). A number of chemists have actually synthesized a preponderance of one antipode of certain asymmetric molecules using circularly polarized ultra-violet light (Florkin, 1960)

so that the general theory is probably correct. It is in keeping with the thesis that asymmetry begets asymmetry, though it is noteworthy that some workers have resolved racemic mixtures by means of symmetrical agents (Ferreira, 1953).

In any event the efficient cause of optical purity in biological materials presents no problem in principle, but only in the details of its progressive improvement during evolution. The results are encoded in the genome (p. 385) and do not constitute a present physiological problem but a genetic evolutionary one. The physiological problem of present individuals is simply to maintain the optical purity presented by the genetic templates.

Even the higher plants and animals, in fact, produce a certain amount of the unnatural antipode, while most microorganisms produce much more; perhaps this is a measure of their primitiveness though a number of them use the unnatural form for chemical offence and defence against other organisms. In general the unnatural form is excreted or broken down as it is formed (Krebs, 1935), and this explains the apparent paradox that in mammals D-amino acid oxidases are more active than those oxidizing the natural L-forms. In the laboratory a pure preparation of an optical isomer spontaneously racemizes so that *in vivo* also there must be a steady loss of the natural isomer after its formation. This is one probable reason why metabolic turnover is so rapid (p. 187), though it should be realized that optically inactive metabolites such as the fatty acids are caught up in the general flow. In mature somatic cells DNA has a very low rate of turnover (p. 188) and so may suffer a gradual racemization, with corresponding effects on the whole metabolism of the cell, and Kuhn has suggested that this is an important factor in senescence (p. 27). There is a turnover of DNA so long as cells are growing and dividing, and some tissues regularly replace post-mitotic cells but there is no replacement in the most important of all tissues, the nervous system. Here senescence is particularly evident and critical; whether it is due mainly to recemization of optical isomers remains to be shown.

There is also the problem of the "final" cause of optical purity, the biological advantage of purity over the racemic state. Mills (1932) pointed out that if a catalyst has a greater affinity for one antipode then the number of fruitful encounters with substrate molecules will increase with the optical purity of a solution of given concentration. In the extreme case of enzymes, with their reaction-specific groups themselves attached to asymmetric carbon atoms, and consequently with an almost absolute specificity for the one antipode, the rate of reaction may be twice that with a racemic mixture of the same concentration. The amount of active enzyme is probably quite as often rate-limiting as the concentration of substrate, so that the rate in biological systems may be as much as four times that between a racemic substrate and a racemic enzyme. It has been shown (Needham, 1959) that speed of metabolism *per se* probably has selective advantage.

Other advantages of a similar kind can be visualized. Large polymers of optically pure monomers can grow much more rapidly, and larger, than

racemic ones and the product is more stable (Wald, 1957). There seems to be adequate evidence that optical purity has positive selective advantage, and that it has not developed simply because there would be a spontaneous tendency for preponderance to increase progressively in cyclically operating metabolic systems.

If the evolution had been purely a spontaneous process of this kind, and supposing that some natural asymmetry is the basis of the initial preponderance, then any particular compound should always show the same antipode preponderant in all organisms. In fact, however, laevo-turpentine is found in American trees and the dextro form in Europe. Indeed within one continent, Australia, some species of *Eucalyptus* produce the D-form and others the L-form of their essential oil. The two, in fact, may be produced in different parts of the same tree (Read, 1935). Of course these oils are incidental products rather than key metabolites but they show that living systems have acquired the ability to resolve racemic compounds and to exploit either antipode.

This is relevant to the further important question whether the one antipode has an intrinsic or absolute advantage over the other. The greater biological activity of the natural adrenaline, nicotine, and other agents is not unequivocal evidence. It is certain only that these are the antipodes which react most strongly with other optically pure substance in the body. If the preponderant antipode of these in general has been determined by local, terrestrial asymmetries, then on other worlds the other set of antipodes might be the active set in living organisms. The two antipodes differ little in their energy of formation (Sevag, 1951), as in most other laboratory properties, and it is not certain that the physiological difference is critical. For instance although antibiotics (p. 287) often contain components with the unnatural asymmetry, as though to "jam the works" in the metabolism of an enemy species, there is not always any great change in their virulence when the natural antipode is substituted (Katchalsky *et al.*, 1955). The general conclusion seems to be that there is an advantage in optical purity over the racemic condition, that spontaneous preponderances, therefore, have been exploited, that, in consequence, one specific antipode is usually the active one metabolically but that there is no other, absolute virtue or peculiarity in that one: living organisms can exploit either, or both on occasion.

## CHAPTER 16

### *The Energy Supply for Growth*

THE events of growth have now been followed down to the molecular level of biosynthesis and here it becomes particularly clear that a supply of energy is indispensable for every act of building up: virtually every anabolic reaction is coupled with an energy-releasing, catabolic or respiratory reaction. This is as much an essential component of growth as the anabolic reaction itself and so the question of energy supply may usefully be considered at this point.

It might reasonably be contended that this energy supply is one of the factors controlling growth, particularly since even the raw materials for biosynthesis are placed in that category (p. 265). Indeed Part II of the book might be regarded as beginning here rather than at the next chapter. In defence of the arrangement actually adopted it may be said that much of the interest in the energy supply is of the same narrative character as the treatment in preceding chapters. However, since a descending order of magnitude was adopted (p. 7) for the narrative and an ascending order for the control mechanisms, we pass directly from the one to the other at the lowest level, in the present chapter. Like the building materials and some other factors, the energy supply becomes evidently growth-controlling only when it is made to be a limiting factor; otherwise its behaviour is more of narrative importance.

Two main aspects of the problem of energy supply may be distinguished, the mathematical, dealing with efficiency and balance sheets, and the biochemical, dealing with the actual reactions which yield energy.

#### **16.1. The Efficiency of Growth**

With minor exceptions the growth processes are endergonic (p. 7), the energy so stored enabling the animal to "do work on" its environment in the various ways essential for survival. The leading question (Rubner, 1908) both in theory and in practice is: what is the efficiency of these processes? How much of the energy put in is retained as useful potential energy of the fabric? On the credit side might be placed not only the bare fabric synthesized to carry out the various work functions but also reserve materials to be used as fuel, or to form products such as eggs and milk, as well as those such as mucus, which are released continuously without storage. It is a matter for consideration, which should be included for any particular purpose.

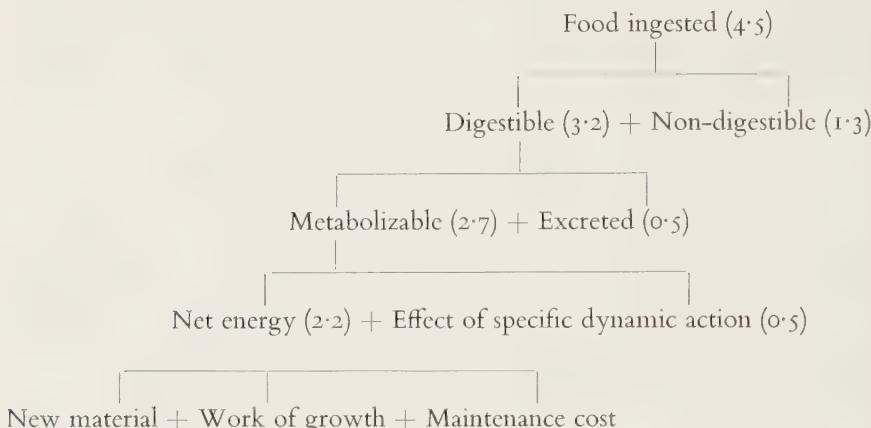
In principle it is not difficult to measure the energy content of the body materials by burning the animal in a calorimeter, and in practice a good

approximation can be obtained, without sacrificing the animal, by weighing it, since the percentage composition of the average body is known, and also the energy value of a unit weight of each component. The energy content of food may be found similarly and the ratio of the increase in energy content of the body to this gives an index which may be called the *crude gross efficiency* of growth, the percentage of the ingested energy stored as body fabric. This proves to be as low as 3 per cent for certain nitrogen-fixing bacteria, which must build up protoplasm all the way from simple raw materials, and as high as 60 per cent in the embryos of some animals; here, prepared food, almost ideal in composition, has been provided by the parent, who has already paid the cost of synthesis. Values as high as 60 per cent are recorded also for a number of marine ciliary feeding animals (Jorgensen, 1952); here the food organisms have paid the cost of synthesis.

The gross efficiency has the virtue of simplicity and for the stockbreeder it is the real interest, since it is the ratio of his returns to his outlay. The ratio is often calculated directly from the wet or the dry weights, since the composition of food and flesh tends to be generally similar, so that it is the simplest possible measure. The ratio is logically unsatisfactory, however, since it charges against the growth account every loss or expenditure of energy. One of the largest items unfairly charged is that of *maintenance*, the energy required to keep the animal alive at constant weight. When this condition of constant weight is imposed on a young animal its gross efficiency of growth must be zero, although it may be carrying out its work and its size-maintenance reasonably efficiently. The gross efficiency of a normal adult man likewise is zero, although again work and maintenance can be highly efficient. The *net efficiency*, therefore, is a more satisfactory index: this is the energy added to the body as a percentage of the total energy available after the deduction of that used for maintenance. Maintenance includes two components, the energy spent in operating the organs and that spent in repairing the wear on them. It could be argued that repair is a growth process, in which case its energy value should be added to both numerator and denominator of the net efficiency ratio; provided it is not too great it will then make little difference to the ratio, and since in practice it is difficult to distinguish it within the maintenance item, it can usually be neglected. There is some variation in the amount of wear with the amount of work, so that in any case the item is partly chargeable to the cost of work and only partly to growth.

The net efficiency of the chick embryo is as high as 97·5 per cent (Wetzel, 1944), compared with a gross efficiency of 38 per cent (J. Needham, 1931, p. 496). In the trout, at the age of two years, it is still as high as 75 per cent (Brown, 1946). Measured in this way the index is still a little unrealistic, however. On the debit side, in the denominator, there are items which could be charged to *general inefficiency* but not legitimately to the inefficiency of growth itself. Some of the food ingested fails to be digested, or is lost to gut fauna, and some is excreted unused, after absorption. Of that metabolized

some is squandered owing to the unharnessed acceleration of metabolism caused by the food itself, an effect known as *specific dynamic action*. The remainder, known as *net energy* is about half of that ingested and is used for weight increase, for the actual *work of growth* and for maintenance. It may be precisely the fraction which takes part in metabolic turnover (p. 187), since it is about the same percentage. Estimates have been obtained of the value of some of these items (Brody, 1945), and a partial analysis, with the energy in large calories (kcal) per gramme of initial food material, may be given as follows—



Rubner appears actually to have used net energy and not food ingested as his measure of energy put in, so that he obtained higher values for what may be called the *true gross efficiency* than those obtained using the crude ratio considered above. The corresponding *true net efficiency* is simply: (new material)/(new material + work of growth) and appears to be essentially what Hofman and Lees (1952) call the *free-energy efficiency*, that amount of energy going into the reactions of synthesis which is retained in the fabric constructed. Provided that the actual energy contents and not simply weights are measured this is also identical with the *net thermochemical efficiency* of Mayer *et al.* (1951). Mayer points out that there is a large potential error in using weight as the measure of energy content. In mammals there is a sudden switch at maturity from protein construction to the deposition of fat, having twice the energy content per unit weight. The efficiency of the weight increment decreases sharply at that time but that of the energy increment, the net thermochemical efficiency, remains virtually constant.

In order to complete the analysis above it is necessary to measure the two items, work of growth, and maintenance cost. A reasonable measure of the two together is given by the heat output of the animal (neglecting specific dynamic action) or more indirectly by oxygen consumption, the calorific value of which is known. Both heat production and oxygen consumption

per unit weight are higher in the growing animal than in the non-growing adult (Wetzel, 1944), and vary directly with the growth rate, all three showing a marked increase at the onset of puberty in a mammal (Brody, 1945). From the results of Collier (1947) on individual, fasting *Tubifex* worms (no specific dynamic action, therefore), it may be computed that during 13 days of regeneration the oxygen consumption per unit weight shows a mean excess of 43 per cent above the normal value for non-regenerating worms. This increase, therefore, must be due entirely to the work for new growth. The loss in weight under the two conditions would provide a cross-check but unfortunately it was not measured on precisely the same group of animals. A 43 per cent increase in oxygen consumption during regeneration indicates that maintenance is still using 70 per cent of the energy dissipated; the figure from weight losses is 55 per cent.

Rubner obtained a value around 35 per cent for his index of gross efficiency during the doubling of the birth weight of various animals. With the value 2.2 kcal for the net energy this gives 0.8 kcal per gramme of food as the energy content of new material and 1.4 kcal for the work of growth plus the maintenance cost. This last item would be between 0.77 and 0.98 kcal in regenerating *Tubifex*, depending on which of the two indices above is used. The work of growth, therefore, would be between 0.63 and 0.42 kcal and the net efficiency between 56 and 66 per cent. This range overlaps that already quoted. Goddard (1948) gives 40 per cent as the maximal fraction of respiratory energy spent on the work of growth and this again would give values of 61 per cent or better for the net efficiency. They are, of course, not so good as the best values for embryos.

*A priori* it would not be surprising if the value obtained in this way for the whole process proved lower than those for the efficiency of the individual chemical reactions of growth, but in fact it appears to be at least as high (p. 258). In addition to the energy cost at the molecular level there must be work expended in moving materials, in dividing cells and in systemic activities. However, the last item, at least, will be submerged in the general maintenance cost and this may help to account for the high values found for the net efficiency of the whole animal. Embryologists have distinguished also the components of energy expenditure due to cell movements and to differentiation (J. Needham, 1931) and have tentatively estimated them at 4 per cent of the total expenditure. Some of these processes may be reversible, and the energy therefore stored, so that, like growth, they contribute to the credit side of the growth account, though this particular gain in potential energy would not be reflected in a weight gain. The energy released by the irreversible processes in this component is probably small enough to be ignored (Waddington, 1959), though it would be interesting to have actual values for it, as for all components. The incorporation of amino acids costs about 1.0 per cent of the respiratory energy used by the cell (Sacks, 1953, p. 215) and a rather similar value is calculated for the maintenance of the body's proteins in their steady state (von Bertalanffy,

1960). Much the same value again is found for the extension of the wall of plant cells (Frey-Wyssling, 1948). At this rate there might be around 20 to 40 components of growth at work in a cell.

The advantage of using net energy rather than total intake as denominator is illustrated by the fact that the specific dynamic action varies with total intake, in the cow, for instance, rising from 3 per cent of the gross energy at a dietary level of half the maintenance requirement, to 20 per cent at maximal intake (Brody, 1945). Even if none of the other items of wastage varies greatly under natural conditions, net energy is likely to be a rather variable fraction of the gross energy, and to complicate the comparison between species, which is a major object once a reliable index is found.

By contrast the energy content of new material seems to be a rather constant fraction of net energy. Not only this, but also the maintenance item is relatively constant, so that Rubner's true gross efficiency proves almost as useful as net efficiency, though not more so, since it follows that the work of growth also must be a relatively constant fraction. In his classical work Rubner (1908) found that the value, 35 per cent, for the gross efficiency of doubling the birth weight was valid for all animals tested, except man, irrespective of size (Kleiber, 1936) or of taxonomic position, a specific energy requirement of 4,000 to 4,800 kcal per kg of material added. The gross efficiency of productive activities is around the same value, 33 per cent for milk yield (Brody, 1945). The kitten doubles its birth weight in eight days and the pike in 270 days, but both have the same gross efficiency; moreover the one is homoio- and the other poikilo-thermic. Hanko (1948) found gross efficiency independent of temperature and other variables.

Although gross efficiency must fall to zero at adult size (p. 253), and, therefore, might appear to be a continuous variable during ontogenesis, it is in fact, very constant over much of the growth cycle. Moreover, a number of the variables which confuse interspecific comparisons of efficiency are relatively constant within a particular ontogenesis, so that the pig, for instance, gains 0.18 to 0.36 g dry weight per gramme of food over a long period (Goddard, 1948). Even the crude gross efficiency, therefore, is constant over much of the individual growth cycle. From this it is clear that growth and metabolism in general are closely interrelated, and that the present aspect of the subject supports the idea of an integral life programme (p. 27). Moreover, it appears that the programme has much the same character in all animals. It is further significant, therefore, that the total heat expenditure per life span, per unit weight, also is very constant in all animals, except man. This means that the short-lived have a high metabolic rate and that the fabric of all can perform a fixed amount of work before it is exhausted, or that it can be renewed only a limited number of times in the repair of wear. The hearts of the mouse and elephant beat approximately the same number of times during their very different life spans (p. 27).

The gross efficiency of man is anomalous but his net efficiency is in the

normal range and this is one reason for preferring this index, for some purposes at least. Man is outstanding in having shifted his growth/work ratio in favour of the latter. Only species with well-protected young could afford to do this. The high work/growth ratio is maintained after the adult size is reached, that is to say, the wear on the working fabric is very economical and so he is very long-lived relative to his rate of activity. He appears not to have been forced to pay the price which might have been expected for the increasing activity which his enlarging brain prompted. His young have leisure to acquire much work experience while the fabric is still plastic enough to make good use of it.

Hybrids typically have a high growth rate and a bigger final size than either parental pure line (p. 386) and this is correlated also with greater efficiency in food utilization (Dickerson, 1954). Experimental selection for rapid growth proves to be a selection for greater efficiency. It seems likely, therefore, that the normal, very similar efficiency in so many animals is the result of some universally relevant force of selection. It is important to remember that "efficiency" may be simply the measure of competition between growth and the work functions: it is no use growing rapidly if the animal has no energy to defend itself or to seek food, or to gain experience. Similarly a larger adult size results in a larger item of maintenance, which also could be a liability. These are not problems which worry the stockbreeder, however; for him time is money, his animals are cosseted, and the slaughterhouse solves the problem of adult maintenance.

To summarize—

Estimates of the efficiency of growth have been confused by the use of a variety of indices. If measured in a consistent way the efficiency of growth has much the same value in all species, irrespective of life span, size, body temperature, and so on. Gross efficiency is 30–35 per cent and net efficiency 60–65 per cent, and the processes themselves are probably very similar in all. The individual items of the energy balance sheet vary less than might have been anticipated and most indices of efficiency are useful for comparative purposes. For absolute measurements choice of index depends on the motive for making the measurements, but more standardization would be a great advantage. The gross efficiency compares favourably with that of non-living systems. The similarity between the values of a particular index in various types of animal supports the idea (p. 27) that the life-programme is an entity which is much the same in all animals. Man alone seems anomalous, in his very slow growth and his long life.

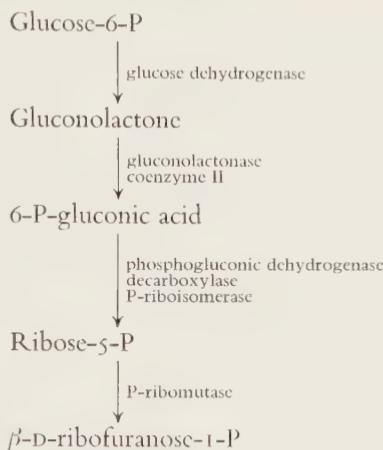
## 16.2. The Source and Manipulation of Energy

All the common materials which could be used as fuel, carbohydrates, fats,

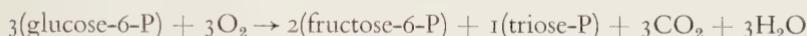
and proteins, are actually used at some time by growing animals: probably all can be used for the work of growth itself. The details of the pathways by which they are broken down in normal metabolism are given in the standard textbooks (Baldwin, 1953); for the present purpose it is adequate to note that all three types of fuel feed into the Krebs TCA cycle for their final main phase of oxidation, and that all the fats and carbohydrates, and some of the amino acids enter the cycle as active acetate (p. 313). The remaining a.a.s feed into the cycle at other points (Krebs and Kornberg, 1957; Kit, 1960). There is some preliminary oxidation of carbohydrate before entering the cycle; this is known as glycolysis, and may be aerobic or anaerobic.

Glycogen is broken down via hexose mono- and diphosphates to the triose phosphoglyceraldehyde, which is further phosphorylated and oxidized to diphosphoglyceric acid, subsequently dephosphorylated and converted to pyruvic acid. Under anaerobic conditions the pyruvic is reduced to lactic in order to reoxidize the coenzyme I (p. 308) required to oxidize more glyceraldehyde; under aerobic conditions coenzyme I is reoxidized aerobically and the pyruvic acid is oxidatively decarboxylated to active acetate. Oxygen normally induces the switch from glycolysis to complete oxidation, the so-called Pasteur effect. There is a limit to the accumulation of lactic acid which can be tolerated, but local glycolysis is possible if the acid is removed for oxidation or resynthesis elsewhere. As Baldwin points out the efficiency of energy release in glycolysis *per se* is not greatly inferior to that for complete oxidation and its main drawbacks are the large amounts of sugar required, the toxicity of the lactic acid and the further expenditure of energy if it is resynthesized to glycogen. Three energy-rich  $\sim P$  bonds are generated when one glucose residue from glycogen is degraded as far as lactic acid; this is equivalent to as much as 34,500 calories per gramme molecule of glucose. The total decrease in free energy is 57,000 calories and the efficiency, therefore, is 61 per cent. However, if one-fifth of the lactic acid is completely oxidized in resynthesizing the remainder of glycogen this represents an energy expenditure of 137,000 calories per mole of original glucose and the efficiency then is only 25 per cent. By contrast complete oxidation of a mole of glucose yields 686,000 calories (Baldwin, *l.c.*) and 38  $\sim P$  bonds per glucose residue are generated, so that the maximal efficiency, at 11,000 calories per gramme equivalent of  $\sim P$  bonds, is 67 per cent. Aerobic respiration is, therefore, the pathway of choice, if  $O_2$  is available; there must be other reasons (*see p. 237*) why rapidly growing tissues use glycolysis extensively. A recent estimate (Burton, 1958) is that the average energy value of the  $\sim P$  bond is only about 8,000 calories per gramme equivalent, which would necessitate a proportionate reduction in all the efficiency values quoted; these are already near the lower limit of the range of net efficiencies shown by the whole animal (p. 255).

Galactose and other storage forms probably all feed into the glycolytic system (Florkin, 1960). A certain amount of glucose is normally degraded to pentose via the gluconic acid shunt—



This pentose can be oxidized and decarboxylated to triose, yielding energy; the overall reaction is—



This is obviously a more fully aerobic pathway than the glycolytic sequence and yields a correspondingly larger amount of energy. Ribose-1-P for the synthesis of nucleic acids (p. 225) is formed by an extension of this pathway, as shown above.

In the use of fats there are probably no important alternative pathways to the main one of  $\beta$ -oxidation (Baldwin, 1953). This involves the repeated removal of a  $\text{C}_2$ , active acetate, unit from the carboxyl end of the molecule of the fatty acid, directly coupled with its entry into the Krebs cycle. The process is a transacetylation from the fatty acid to oxaloacetic acid, forming citric acid which is the starting point for a cycle of oxidation. Each oxidative removal of an acetyl residue provides enough energy to synthesize five ATP molecules from ADP (Florkin, 1960) and the further oxidation of the residue provides for twelve more, so that stearic acid ( $\text{C}_{18}$ ) can yield—

$$(8 \times 5) + (9 \times 12) = 148 \sim \text{P}$$

compared with 114 for three glucose ( $3\text{C}_6$ ) molecules. The total energy liberated by oxidizing one molecule of stearic acid is about twice that from an equal weight of carbohydrate, three glucose molecules being considerably heavier than one stearic molecule.

Growing animals may have no unique respiratory mechanisms but they do exploit some of the less common pathways more extensively than adult animals. This applies equally to rapidly growing tissues even in adults (p. 129). Glycolysis is very active at certain, particularly the early, stages of development (J. Needham, 1942) and the Pasteur effect is usually slight, so that glycolysis continues

aerobically. The gluconic acid shunt is often very active (Boell, 1955), probably because of the extent of nucleic acid synthesis.

A high rate of glycolysis is characteristic also of neoplastic growth (p. 98), and some anticarcinogens specifically inhibit glycolysis (J. Needham, 1942, p. 265). The rate of glycolysis in the different regions of the body also is related to their rates of growth. It is three times as high in the dorsal lip of the blastophore of the vertebrate embryo as it is elsewhere, and it is high in the epiphyseal region of bones (Eeg-Larson, 1956). A correlation between glycolysis and protein synthesis also has been detected (Cuthbertson, 1958), and it may be significant in this connexion that the pseudopeptide bond in ornithuric acid can be formed under anaerobic conditions if ATP is provided as a source of energy (Cohen, 1951). At one time it was thought that glycolysis was used by embryos because their oxygen supply was not adequate until the circulatory system was fully established, but in fact glycolysis rate often increases progressively during embryonic development, in parallel with circulatory improvements (S. Smith, 1957). It continues aerobically, in fact (Harris, 1958). The low oxidation potential associated with glycolysis is probably favourable to the endergonic reactions of biosynthesis, but the more specific reason for this pathway of respiration (p. 237) seems the most important. A number of amino acids and other metabolites are synthesized directly or indirectly from the Krebs cycle.

It seems likely that the gluconic shunt also can function in the same way, as an alternative to the Krebs cycle, when this is operating in the direction of synthesis. If so there is the question what decides between the glycolytic and the gluconic pathways? One possibility is that the latter is used: (1) when nucleic acid synthesis is rapid and (2) when a rather higher oxidation potential is advantageous. Cell division is a possible occasion of this kind, but in fact there is considerable evidence (Ørstrøm and Lindberg, 1940; Boell, 1955; Harris, 1958) that relatively reducing conditions are necessary for cell division (p. 338); for instance, reducing conditions are found in young proliferating meristem tissues (van Fleet, 1954), whereas a distinctly higher oxidation potential prevails in the region where growth is by cell enlargement only. Weber (1958) has shown that there is much similarity between cell division and other motor activities, such as muscle contraction, and this also is anaerobic in its most active phase. In barley seedlings mitosis continues for some days under anoxic conditions (Hughes, 1952a, p. 188), and *in vitro* animal cells continue to divide in high concentrations of cyanide (Willmer, 1953, p. 80). Oxygen consumption spontaneously declines each time the cell cycle approaches division (Aronoff and Graf, 1953). Antioxidants protect the nucleus against the oxidizing and antimitotic action of ionizing rays (p. 421), while biological reducing agents, particularly —SH compounds, are present in high concentrations in dividing cells (Mazia, 1956). One aspect of this function may be to prevent the oxidation of deoxyribose.

On the other hand, it seems that some phases, at least, of the division

process are aerobic (Swann, 1952; Ris, 1955; Boell, 1955), and among anti-mitotic agents there are many (Table 21.2, p. 345) which inhibit the action of the cytochromes as terminal oxidases. If the formation of the spindle is anaerobic its subsequent resolution is not unlikely to be aerobic (p. 142). Equally, however, terminal oxidation plays a part in growth processes proper; the more endergonic the synthesis the more (exergonically released) energy it may require. A yeast mutant lacking the cytochrome system proved to have an abnormally low growth rate (Spiegelman and Sussman, 1952). In some insects investigated a temporary inhibition of this system marks the onset of diapause, and its activity returns as the first step in the resumption of growth after diapause (J. Needham, 1942; Williams *et al.*, 1952). An artificial diapause can be induced in such insects by the respiratory inhibitor carbon monoxide (Wigglesworth, 1954*b*, p. 44). There is a 200-fold increase in respiration when seeds begin to germinate (Goddard, 1948), and only the early stages will proceed anaerobically. It is often difficult to decide whether the action is primarily on the phase of growth or on that of cell division (p. 341), but in the oocyte (p. 129) for instance there is only synthesis, and no division.

Induced enzyme synthesis (Spiegelman, 1950) and protein synthesis in general (Voegtlín *et al.*, 1933; R. Brown *et al.*, 1952; Jones and Bonting, 1956) require oxygen, and so does the incorporation of  $^{32}\text{P}$  into RNA (Strickland and Rossiter, 1953). This is also true for amino acid incorporation (Peterson and Greenberg, 1952) and even for the initial fixation of nitrogen by autotrophes (McLean and Fisher, 1947). Agents which uncouple phosphorylation from terminal oxidation regularly inhibit growth (p. 375). As already suggested (p. 258) aerobic respiration is to be expected wherever feasible, on grounds of efficiency.

There is evidence that the various inhibitory agents for growth act differentially on terminal respiration, glycolysis, and growth itself (J. Needham, 1942), and this indicates that the latter is not absolutely tied to either level of oxidation. The interpretation is necessarily complicated by the fact that growth rarely commands more than 40 per cent of cellular respiration (p. 255) and much less of that of the whole animal. Considering all the evidence it seems probable that the early stages of development, with rapid cell division, may benefit from a low oxidation potential whereas later stages, dominated by extensive synthesis and other components of growth, with more cell-hypertrophy demand a higher potential, with terminal oxidation.

The second major problem concerning the energy supply for growth is the sequence of substrates used by embryos and the reasons for the sequence. A carbohydrate-protein-fat sequence in the chick (J. Needham, 1931) has been confirmed for the grasshopper embryo (Boell, 1955), but aquatic animals, echinoderms, fish, and Amphibia have different patterns. In the trout (S. Smith, 1957) fat is used first, then protein and carbohydrate and finally fat again. Fats are the most fully reduced of the three classes of fuel, and they must be oxidized aerobically for that reason; therefore, according to conditions, they

may or may not have advantages in early development. Protein cannot be used unless its waste products can be eliminated or inactivated. The trout needs an abundant oxygen supply. The sequent changes during animal ontogenesis are not necessarily due to exhaustion of the previous substrate, as the later return to fat utilization in the trout shows, and so they may reflect changes in the character of the growth itself (Boell, *l.c.*). Protein is sometimes used as a source of energy even before it is extensively used for synthesis. This would seem a serious risk in the eggs of most animals, since they are provided with limited protein nitrogen, and it must carry some overriding advantage. Variations between species may be due to differences in the composition of their egg yolk or in their developmental requirements, and it would be useful to know if yolk composition is in fact a specific provision for the latter; this is an important field for further study.

The mitochondria bear the complete system of enzymes concerned in aerobic oxidation of Krebs cycle substrates, and it has, therefore, been called the cyclophorase system (Green, 1951). The mitochondria may solve a critical problem, that of oxidizing substrates aerobically without causing an excessively high oxidation potential throughout the cytoplasm. In many cells they move around actively, probably catalysing oxidations locally but leaving a lower potential elsewhere. In bacteria they are associated with the cell membrane, where the oxidation potential is likely to be highest. They often crowd round the equator during the division of animal cells, and this also might be a useful localization of oxidizing conditions.

It seems likely that there are a number of ways in which respiration might control the rate and the character of growth, quite apart from any question of absolute shortage or abundance of respiratory substrates. The ability to use different substrates and different pathways according to internal circumstances (themselves not yet fully understood) illustrates the kind of possibility. In one case at least, the arrest and subsequent resumption of growth in the diapause cycle of insects (*p. 426*), the respiratory systems appear to be the critical, proximate, controlling factor. Some of the hormones controlling growth in both insects and vertebrates act through the respiratory systems. There is already enough evidence to justify the claim made at the beginning of the chapter that the energy supply is as important as the anabolic reactions themselves.

PART II

*The Control of Growth*



## CHAPTER 17

### *The Raw Materials for Growth*

IN pursuance of the general plan (p. 7) growth-controlling agents will be studied in order, according to the level at which they act, beginning with the lowest level, that of the constituent reactions of biosynthesis. The scheme has its difficulties; thus some agents may control at more than one level. Raw materials are initially the concern of the body, as a feeding organism, and so act as a systemic control. Consequently the size of the gut in planarians is a controlling factor in their growth rate; only after digestion and assimilation do the materials control growth directly, at the molecular level. In general, however, each type of agent is found to act at one particular level, so that the present treatment gives a reasonable classification also of the agents themselves: according to convenience, therefore, the chapter headings emphasize either level of action or class of agent. Of course, any effect at one level is likely to have manifestations at all higher levels, and these are often the best known, because most easily observed. Sometimes they are, at present, the only evidence available. In any case they are essential parts of the complete picture of the growth mechanism, and will be recorded where this is most convenient.

The order of treatment is: the raw materials or substrates for synthesis, the enzymes catalysing the reactions of synthesis, the control of the energy supply for these reactions, nucleic acids as special agents in intracellular synthesis, morphological intracellular agents, controls at the level of cell division, the agents controlling the growth of tissues and organs locally, and finally systemically acting agents, including genetic control and external factors. Since, strictly speaking, growth is a quantitative phenomenon, only its speed can be affected by these controls and, therefore, interest centres on the way they control growth rate. However, qualitative effects may appear because growth is normally differentially distributed (pp. 2, 31) and because the agents may also add their own differential effects.

Raw materials control growth in two main capacities, as constituents of the growing fabric itself and as components of enzymes and other agents of internal control. In self-regulating systems such as living organisms it is often difficult to distinguish sharply between the two roles; usually some, at least, of the compounds into which an element is incorporated then become agents in the control of further growth. It is particularly important to distinguish between the two roles where possible, therefore. For the purpose the term *fabric* includes not only flesh and bone but also body fluids, hormones, enzymes,

and all other components significant in the functioning of the body; it is not a purely morphological term.

### 17.1. Water

For animals water is the simplest of the raw materials but its great and fundamental importance can scarcely be overemphasized. Five main types of function may be recognized for water: (1) purely mechanical, as in the distension of aquatic arthropods after ecdysis and in the distribution of nutrients and other materials in the circulation; (2) as a physical medium in which living material is dispersed and in which all growth processes and movements can proceed in orderly manner; (3) osmotic, as in the maintenance of cell turgor and in the acquisition of mineral and other nutrients (Kenyon, 1940), the cells, in general, having a higher osmotic pressure than the body fluids (Bartley *et al.*, 1954); (4) as a chemical reagent in growth processes, as in photosynthesis and in hydrolyses; and (5) as a component of the fabric itself, as water of hydration and bound water. According to some views water other than bound water also may be an essential part of the living fabric, forming a hydrogen-bonded continuum with proteins, nucleic acids, and other components (Jacobson, 1953). The first three might be regarded as agent-functions and the last two as primarily fabric-functions.

Egg cells, in general, have a high ratio of solids to water, that is they are rather desiccated and absorb much water subsequently, from the medium or from an albumin solution. The dehydrated condition economizes weight, but this is not its only virtue since the sauropsid vertebrates cancel any such advantage by surrounding the cell with a large mass of albumin solution, and the completed egg is particularly bulky. More important is the general inertness and stability of the dehydrated condition (p. 114). The extent of the initial deficit of water is well seen in the frog embryo, which swells as much as 14·5 per cent even during cleavage and continues to absorb water subsequently, so that during the first ten days after hatching it increases ten times in weight, almost entirely by water intake (Davenport, 1899, p. 285). In the next thirty days this weight is again multiplied by ten, about 90 per cent of the increase being due to water. The human embryo grows to 50,000 times the size of the egg in six weeks, and 98 per cent of this is water (Davenport, 1936); here the egg is virtually yolk-free and water is assimilated simply because it is the major vital constituent of the young embryo.

Indeed, a high degree of hydration is essential for growth, and animals are maximally hydrated at an early stage—in fact, as soon as they have diluted the egg contents. In the chick maximal growth rate corresponds to the time of maximal hydration (J. Needham, 1942, p. 69). Water content decreases throughout life in man (Parker, 1958), particularly the extracellular water, but those fishes, which grow throughout life, maintain their hydration (J. Needham, 1942, p. 541). Even in bacteria (Richards, 1934) old cells are relatively dehydrated (p. 114) and phases of growth-arrest, such as encystment and

diapause (pp. 427, 425), are preceded by water elimination. Reciprocally the onset of a new period of growth in tissues of the reproductive system is heralded by increased water uptake, promoted by the gonadal hormones (Roberts and Szego, 1953). Hydration improves the rate of regeneration (Needham, 1952, 1960), and regenerating pieces of planaria take up extra water (Krogh, 1938, p. 37). Neoplastic tissues are more hydrated than normal. Anions increase the hydration of proteins, by removing cations which displace water of hydration (Bull, 1943); anions are, therefore, found to cause swelling and to promote growth in consequence (Knase, 1933).

Specialized animals, living in very saline media, have come to grow best at high salinities, and in the brine shrimp, *Artemia*, the growth rate is proportional to a high power of the density (Weisz, 1946). Animals live and grow in waters as saline as the 36·8 per cent of Gus Gundag. Others manage to grow in waters with high concentrations of other salts (Allee *et al.*, 1949).

The state of aggregation of the water may be important (Barnes, 1937, p. 133): water condensed from steam, and, therefore, in a state of low aggregation, does not promote growth as well as melted ice, tested at the same temperature. It would be necessary to ensure that the latter contains no extraneous promotive agents which would have been destroyed at 100°C in the other sample.

Deuterium oxide, heavy water, probably stimulates growth in low concentration but inhibits in larger dosage (Barnes, *i.c.*). This is the characteristic action of deleterious agents. However, toxic effects are evident only above a concentration of 20 per cent or so. More than 75 per cent is necessary to inhibit cell division (Gross and Spindel, 1960); the action is prompt and is equally promptly reversed on return to H<sub>2</sub>O. It is thought to be simply mechanical, through the increase in viscosity.

## 17.2. Inorganic Elements

The distinction between organic and inorganic elements is somewhat arbitrary, and is not simply a distinction between those which do or do not enter the fabric of the body. Some which are usually regarded as typical inorganic elements, potassium, iron, cobalt, copper, and iodine form definite compounds with organic constituents, and are essentially part of the fabric. On the other hand phosphorus is usually included among the organic elements, though it is always in the simple form of phosphoryl and phosphate (p. 245). The distinction is not rigid quantitatively either: elements such as calcium, potassium, sodium, and chlorine, although present mainly in inorganic form, are required in large amounts by most, if not all, animals. The percentages of the main elements in man (Hawk *et al.*, 1947, p. 987) are—

Oxygen . . . .	65·0	Phosphorus . . . .	1·0	Magnesium . . . .	0·05
Carbon . . . .	18·0	Potassium . . . .	0·35	Iron . . . .	0·004
Hydrogen . . . .	10·0	Sulphur . . . .	0·25	Manganese . . . .	0·0003
Nitrogen . . . .	3·0	Sodium . . . .	0·15	Copper . . . .	0·0002
Calcium . . . .	1·5	Chlorine . . . .	0·15	Iodine . . . .	0·00004

The distinction between organic and inorganic elements, therefore, is largely arbitrary, if convenient. The organic group, carbon, hydrogen, oxygen, nitrogen, sulphur, and phosphorus, are all non-metals, though several other non-metals, such as iodine, chlorine, and boron, are essential constituents, and necessary for the growth of some, if not all organisms.

The main organic materials based on C, H, O, and N will be considered below (p. 279), and, in fact, most of the book is concerned with their role in growth. The importance of P and S also is evident throughout, and merely requires recognition here. Their functions as fabric material are considered on p. 245; a function of phosphate not emphasized there is its buffering action, in the form of its alkali metal salts. Inorganic phosphate has been found to speed early development (Davenport, 1899) and the later growth of animals (Needham, 1947), as well as the growth of isolated cells (Waymouth, 1954) and of microorganisms (Eddy and Hinshelwood, 1951). It has long been recognized as a major element in plant nutrition. It is highly concentrated in the seeds and at the growing points of plants, and in the egg yolk and milk of animals (Davenport, 1899, p. 313). It is mainly in organic combination in eggs and during development inorganic P is progressively set free (J. Needham, 1931, p. 1237); presumably the rate of regeneration of organic forms does not keep pace with their dephosphorylation in respiratory metabolism and other processes of energy transfer. Some microorganisms store large amounts of polyphosphates, that is orthophosphate polymerized by a series of pyrophosphate type bonds. These bonds are "energy-rich" (p. 245) and the polyphosphates are found to be important for growth (Schmidt, 1961).

Sulphur has long been known as an essential element for plants (Stiles, 1950), and deficiency causes much the same widespread symptoms as those of nitrogen and phosphorus. In growing oats the percentage of sulphur progressively increases (Davenport, 1899). Animal growth depends on an adequate supply of methionine (p. 289) and the B-vitamins thiamine, biotin, and pantothenic acid; this last does not contain sulphur but becomes conjugated with a sulphur compound in its active form (p. 313). Inorganic sulphate has been found essential for the growth of the skeletal spicules of echinoderms (Davenport, 1899). The importance of methionine has perhaps been most clearly demonstrated in wound healing of the skin (Needham, 1960), though there is every indication that it is equally essential wherever sulphur proteins are being formed, since it is the source of S for cysteine and cystine also (p. 242). In the absence of sulphur the growth of *Chilomonas* is slow, fat accumulates and oxygen consumption falls (Mast *et al.*, 1936); the fat accumulation implies methionine deficiency, and the respiratory change indicates a lack of cysteine and of GSH (p. 287). Sulphur forms certain compounds analogous to those of phosphorus, for instance thiomethylriboside (R. L. Smith *et al.*, 1953) and adenyl-L-methionine (Brachet, 1957, p. 280), and —SH behaves to some extent like —NH<sub>2</sub>. A good deal of S metabolism can be interpreted as the action of the sulphonium ion, SH<sub>3</sub><sup>+</sup>, the analogue of ammonium, and a strong

base (Shapiro and Schlenk, 1960). Sulphur helps to protect the body against its poisonous analogue, selenium (p. 276).

The total number of inorganic elements indispensable for growth is very great (Fearon, 1949; Stiles, 1950; Underwood, 1962). Frequently they are required in only trace amounts, and boron at a concentration of  $10^{-6}$  or even  $10^{-7}$  is adequate for the growth of carrots and beans (Warrington, 1940; Quastel, 1947). The demand for many of them was not suspected until precautions were taken to ensure an absolute deficiency. Most elements tested experimentally are found to promote growth, in low concentrations at least (Davenport, 1899; Neipp, 1937; Brody, 1945). It is probable that most of the many elements detected in living tissues (Fearon, l.c.) are retained because they are essential constituents, and so also essential for growth, in one or both capacities. A few, such as lead, do seem to accumulate passively because there is no effective method of excreting them, but the concentration of most is controlled, at the optimum; they can be excreted via skin and gut, even if very insoluble. Plants do not accumulate excesses (Steward and Miller, 1954); they take up inorganic elements in constant ratios, and in absolute amounts proportional to their total growth.

In general there is a negative correlation between the relative amount of an element in living organisms and that in the rest of the biosphere (Fearon, 1949). This may imply that, in general, living organisms are systems making use of the less common elements in the earth's surface or that the amounts of those elements which are continuously locked up in living bodies are adequate to cause a significant scarcity in the rest of the biosphere. In either case it is clear that living organisms select their elements and do not passively accept materials in the proportions presented by the environment.

The significant elements, those which are not casual contaminants, are often divided into macro-, minor, and trace constituents of the body, again for convenience rather than for clear physiological reasons. As part of a functional classification it is useful to distinguish the physiological saline elements, Na, K, Ca, Mg, and Cl, present largely as ions, and in high concentration, and so capable of exerting osmotic and electrochemical effects, in particular, as well as some individually specific effects. It is they which effectively control the amount of water bound to the proteins. A second functional group contains Fe, Cu, Co, Mn, Zn, Va, and Mo, present in micro amounts. They are mainly held in organic combination by coordinate bonds and by chelation (Albert, 1950), while still retaining ionic bonds free for other purposes. Their compounds form the prosthetic groups of enzymes and other proteins, while their free inorganic salts often act as the cofactors of enzymes. They are concerned mainly with oxidation-reduction processes, that is with rH phenomena rather than with the ionic and pH effects due to the first group. This distinction is relevant to their functions in growth. Again the distinction is not absolute: in the first group calcium is a vigorously chelated element while magnesium is bonded to a porphyrin, in chlorophyll, and both are cofactors for many enzymes.

Most of the remaining essential elements are required in such small amounts that a catalytic function of some kind, not necessarily enzymic, must be anticipated. Their function may be as agents in growth, or as components of very special fabric materials with critical roles in metabolism. Iodine, as the essential component of the thyroid hormone (p. 373), is a good example of an element with probably a non-enzymic, promotor action on both growth and general metabolism.

The following are examples of mixtures of inorganic salts used in stock laboratory diets, expressed as percentages of the total weight of salts. The total was usually between 2 and 4 per cent of the total dry weight of the diet. Figures in brackets are the numbers of molecules of water of crystallization.

Salt	Mixture of				
	Hamilton and Hogan (1944) (hamsters)	Sure (1943) (rats)	Gay (1938) (meal- worms)	Hubbell <i>et al.</i> , (1937) (rats)	Phillips and Hart (1935) (rats)
NaCl	6·9	17·4	4·7	6·9	15·0
NaH <sub>2</sub> PO <sub>4</sub>	—	—	9·4	—	—
KCl	11·2	—	—	11·2	—
KH <sub>2</sub> PO <sub>4</sub>	21·2	—	—	21·2	—
K <sub>2</sub> HPO <sub>4</sub>	—	33·4	25·8	—	37·9
KI	0·008	0·10	0·009	0·008	0·07
CaCl <sub>2</sub>	—	0·026	—	—	—
CaCO <sub>3</sub>	12·5	31·1	—	54·3	26·9
CaHPO <sub>4</sub>	—	(2) 9·9	—	—	(4) 8·5
Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	—	—	14·6	—	—
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	37·6	—	—	—	—
Ca lactate	—	—	35·2	—	—
MgSO <sub>4</sub>	(7) 3·3	(0) 5·1	(0) 7·2	(0) 1·6	(7) 9·1
MgCO <sub>3</sub>	2·5	—	—	2·5	—
MnSO <sub>4</sub>	(4) 2·6	—	(7) 0·009	(0) 0·035	(4) 0·031
FePO <sub>4</sub> ·4H <sub>2</sub> O	2·1	—	—	2·1	—
Fe citrate	—	2·9	3·2	—	2·5
CuSO <sub>4</sub>	(5) 0·14	(0) 0·021	—	(0) 0·09	(5) 0·027
ZnCl <sub>2</sub>	—	0·026	—	—	0·022
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub>	—	0·026	—	—	—
Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub> ·K <sub>2</sub> SO <sub>4</sub> ·24H <sub>2</sub> O	0·017	0·021	—	0·017	—

The similarity between the various mixtures in their total percentages of each of the main ions is not as impressive as might appear at first glance, since most are based on previous formulae, modified for particular reasons. However, the ultimate mixtures were based on the composition of well-balanced natural food materials such as milk, and the minor nature of the modifications is a tribute to the suitability of the natural mixture. It is sometimes important to

allot particular anions to particular cations, although once in mixed solution in the gut this cannot be very critical; in the presence of a soluble carbonate calcium will be precipitated even from its soluble lactate, if the pH permits it.

Most of the elements are best considered individually, but much of the significance of Na, K, Ca, and Mg, as growth agents, lies in the balance between them; there is a balance between the monovalents with a stimulatory action on activities in general and the divalents with a generally depressant action, and also a balance between the two members of each pair. Potassium is usually highly concentrated inside animal cells and sodium in the body fluids, though occasionally the relationship is reversed, so that differential distribution *per se* may be more important than any particular distribution. Thus, again, plants have a high intracellular K, but little Na inside or outside the cell. On the other hand each distribution has become highly specific and substitutes are rarely tolerated. Something has already been seen (p. 186) of the balance between K and Na in the control of uptake of materials, and they profoundly affect growth in general; their distribution changes progressively during the growth of animals, K increasing and Na decreasing in the cell. Na in excess, and unbalanced by K, is toxic and retards growth (Heilbrunn, 1952). For animals the Na deficiency in a vegetarian diet is exacerbated by the K excess.

Na is sufficiently abundant in all food materials of animal origin, and in most waters, to obviate any growth limitation due to a deficiency, though a real shortage is experienced in the heart of the large continents, remote from suspended sea spray: in Africa where high temperatures and sweating exacerbate the condition, the large mammals walk many miles to a natural salt lick. This is an interesting example of quality appetite (p. 282) and an intriguing problem in behaviour. There appears to be no very definite upper limit to the salt appetite, and there may be a danger of habituation and addiction (Kaunitz, 1956). Many foods now are grossly oversalted because the "public taste demands it."

In experimental work it was not possible until recently to induce a Na deficiency sufficient to retard growth. An exception is seen in certain luminous bacteria (Harvey, 1952) which demand a high concentration. This must be distinguished from the non-specific demand of *Artemia* (p. 267) for a high osmotic pressure. There is now adequate evidence (Orent-Keiles *et al.*, 1937; Hawk *et al.*, 1947) that the element is indispensable for the growth of mammals, but little retardation is evident until the dietary concentration is reduced below 0·02 per cent, compared with the 0·9 per cent of normal body fluids! Its main function in growth is that already indicated; it promotes the uptake of amino acids and protein synthesis by the nucleus (Allfrey *et al.*, 1955b), a function served by K in the cytoplasm. Na increases the solubility of some amino acids and of many proteins (Cohn and Edsall, 1943).

The optimal concentration of K seems to be very variable, in different organisms, though there is little doubt that it is an essential element for both plants and animals (Petrie, 1943; Thimann, 1949; Eckstein, 1938). Miller

(1958) found the optimum no higher than  $3 \times 10^{-4}$  molar, and Tennant and Liebow (1942) recorded inhibition at normal concentrations, though the growth of tumour tissue was accelerated. A small excess causes a differential growth in length over breadth in leaves (Huxley, 1932, p. 202), and a greater excess is toxic. A rather precise concentration is necessary also for the incorporation of P into P-lipids and nucleic acids, and of S into sulphur proteins (Roberts, 1950). It is a cofactor for a number of enzymes (Long, 1961). Its importance for the uptake of amino acids by the cell has been illustrated by Petrie (1943), and Gale (1953) and its effect on the enlargement of the plant cell vacuole (R. Brown *et al.*, 1952) is probably another example of its control of permeation into the cell. As a raw material it is necessary for the formation of salts with such proteins as haemoglobin. In the cells K is largely ionized, in contrast to Mg and Ca which are mainly in unionized complexes (James D. Robertson, 1957).

Magnesium is abundant in all foods and deficiency has been produced only by careful experimental work. It is necessary for growth and regeneration (Davenport, 1899) and for skeletal growth, in particular, so that deficiency causes demineralization. Deficiency causes a disease known as grass tetany or grass staggers. In animals Mg is used mainly as a cofactor for a large number of enzymes, alkaline phosphatase (Giri and Datta, 1936), phosphoglucomutase, transacetylase (Stern *et al.*, 1950), carboxylases and dehydrogenases. These are all important in growth as well as in general metabolism. Mg also activates the enzymes which phosphorylate the coenzymes of these transferases themselves! It inhibits ATPase, which liberates inorganic P from ATP, so that it tends to direct P energy into more useful channels (Greville and Lehmann, 1943), as activator of ATP kinase (p. 318), in skeleton formation (p. 172) and in amino acid activation (p. 196). It is a cofactor for virtually all kinases and activation enzymes (Long, 1961). The growth of *Aspergillus* is an exponential function of Mg concentration (Lavolley and Laborey, 1939).

In the vertebrates and other animals with a calcareous skeleton, calcium (Irving, 1957) is a major raw material. In the vertebrates a very precise balance is maintained between the Ca and P contents of the skeleton, and this demands an equally exact balance in the plasma. The equilibrium between the two is regulated by vitamin D (p. 301), and by the parathyroid and adrenal cortical hormones; in this connexion it is interesting that a steroid component has recently been found in the parathormone also (Rasmussen and Westall, 1957). Calcium, in association with histone, is required also for the formation of intercellular cement and for the stabilization of cell membranes and proteins rather generally (Heilbrunn, 1952). Its protective power against lead poisoning (Jones, 1938) conceivably may depend on this property of stabilizing proteins. It also stabilizes P-lipids (Bull, 1940). It is one of the few elements which seems innocuous in the absence of all other inorganic elements (Heilbrunn, 1952). It is required for blood clotting but in general is a good solvent for amino acids. Like Mg, calcium is cofactor for a number of enzymes, some of which are important as agents in growth—for instance, acid phosphatase,

succinic dehydrogenase and ATP-kinase. Through the P-mobilizing enzymes it may directly control the Ca/P ratio (p. 179). That its action extends beyond skeleton formation is shown by the fact that it is necessary for the growth of such animals as the mosquito larva (Frost *et al.*, 1936), which has a non-calcareous skeleton. It promotes the efficient utilization of food (Kleiber, 1940), as any major ingredient of the fabric might be expected to do; at a level of 10 mg of Ca per 100 g of food rats ate a total of 456 g, to reach a weight of 146 g, whereas at a level of 430 mg per 100 g of food the total amount of food required to make this weight gain was only 372 g. Mg and Ca are antagonistic in some respects; Mg causes Ca excretion, particularly if the P-content of the body is low, while excess Ca exacerbates Mg deficiency (O'Dell, 1960). Mg is essential in small quantities for bone growth but deleterious in excess.

Zinc has been shown essential for the growth of animals, plants and bacteria (Stirn *et al.*, 1935), and it is found to promote wound healing. It accelerates mitosis (p. 345) and is highly concentrated in the spindle apparatus (Mazia, 1956). It appears to potentiate the action of pantothenic acid in preventing the greying of hair in rats (Carter and Thompson, 1953, p. 305). In some micro-organisms Zn promotes the synthesis of the amino acid, tryptophan from indole and serine (Nason, 1950), and it also speeds the synthesis of the indolic growth hormones of plants (Wardlaw, 1952). Like Ca it decreases the solubility of proteins and may stabilize the fabric; it is used clinically to prolong the action of the antidiuretic hormone and of insulin (p. 369), and this type of action can be important in the control of growth rate.

Zinc resembles Ca and Mg in acting as cofactor for a number of enzymes, carbonic anhydrase, catalase, peroxidase, uricase, phosphatase (Poulson and Bowen, 1952), enolase, aldolase (Quinlan-Watson, 1953) and zymohexase. This is its main significance as a component of the fabric. Some of the enzymes are concerned in the energy supply for growth. Zinc is known to be essential for the actual biosynthesis of carboxylase (Foster and Denison, 1950): there is a general parallel between the amounts of Zn and co-carboxylase, as measured by thiamine content, and the amount of Zn is low in the disease beriberi (p. 309), due to thiamine deficiency. Sway-back and enzootic ataxia are symptoms of Zn deficiency in cattle (Carter and Thompson, 1953). There is some degree of antagonistic action between Zn and Ca (Forbes, 1960).

Copper (McElroy and Glass, 1950), as a growth factor for vertebrates, is important mainly as an agent for the synthesis of haemoglobin, a major constituent of the body, and of other iron porphyrin enzymes. A deficiency of Cu causes a more serious anaemia than that due to a deficiency of iron itself. It also causes a disease known as *leksucht*, salt sick. Copper is necessary for plant growth. It promotes the synthesis of the anthocyanins of plants (Edmondson and Thimann, 1950), and of animal sclerotins and melanins (p. 245); this last may account for its synergism with pantothenic acid in preventing the greying of hair (Singer and Davis, 1950). For the formation of sclerotins

and melanins it is the essential constituent of the critical enzymes, and as a raw material it is important mainly for incorporation into these and other enzymes, tyrosinase, laccase, ascorbic oxidase, uricase, and others (Long, 1961). It is required in a similar combination in the respiratory pigment haemocyanin, in certain molluscs and arthropods (Redfield, 1934, 1950) and in the connective tissue pigment of chilopods (Needham, 1960b). In mammals there are copper proteins in the blood and liver, haemocuprein and hepatocuprein, and complex Cu-Fe-nucleoproteins in some tissues (Dawson and Malette, 1945).

As a raw material iron is required for the iron porphyrin prosthetic groups of haemoglobin, myoglobin, the cytochromes, and catalase, which are also important agents in the energy supply for growth. This may be the reason why Fe appears to be a general promotor of growth, for instance of the embryo of echinoderms, of bone (Amdur *et al.*, 1945), and of cells in general (Willmer, 1953). It increases as much as five times when cells become tumorous (p. 99). It is essential for the synthesis of a number of the B-vitamins (van Lanen and Tanner, 1948), including folic acid (p. 314) which reciprocally is a requirement for the production of erythrocytes (p. 315). Iron is 3 to 10 times more concentrated in the nucleus than in the cytoplasm (Poulson and Bowen, 1952); here it would seem to be concerned with more general and fundamental aspects of growth.

Iron illustrates very well the importance of an optimal concentration, for in excess it has a number of deleterious effects on growth. The property which makes it the most valuable element for oxygen transport and terminal oxidation also causes the oxidation of such biological reducing agents as cysteine, glutathione and vitamin A (McCollum *et al.*, 1927). By promoting the oxidation of cysteine it removes a normal inhibitor of the activity of peptidases and accelerates protein breakdown (Schales and Roux, 1950); perhaps for this reason its uptake from the gut is very limited. Excess dietary iron, in direct contrast to optimal amounts, can retard bone growth and cause rickets. Here the mechanism is not an oxidation but is due to the formation in the gut of insoluble iron phosphate,  $\text{Fe}_3(\text{PO}_4)_2$ , and of Fe salts of phytin (p. 296), so depriving the body of essential phosphate. This is also a potential, if not an actual means of controlling iron uptake, and it illustrates also the critical importance of the correct balance between the different dietary constituents. Glutamic acid (p. 289) holds the balance in the control of Fe uptake.

Deficiency of cobalt causes a disease in cattle known variously as bush sickness, pine, coast disease, wasting disease, slow fever, moor cling, and marasmus, and characterized by emaciation and anaemia. Like copper, cobalt promotes the synthesis of haemoglobin and other iron-porphyrin compounds. The optimal concentration is 0.02 per cent. Excess can lead to polycythaemia, an excess of red blood cells, and to excess of haemoglobin. Its carcinogenic properties and its effect on mitosis (Table 21.2, p. 345) conceivably may be due to a similar effect on the synthesis of iron compounds in the nucleus. As a constituent of the body, cobalt is the key element of a porphyrin-like compound in

vitamin B<sub>12</sub>, cobalamin (p. 315). Like the other bivalent, heavy metals already considered it is an activator of a number of enzymes, arginase, pyridoxal and flavin kinases and zymohexase. Like iron it also activates peptidases and so is deleterious in excess for a second reason.

Nickel, unlike cobalt, has been shown essential for the growth of plants. It is necessary for insulin synthesis and activates a number of enzymes, mostly those also activated by cobalt. Deficiency causes anaemia and emaciation (Frost *et al.*, 1941). Moore (1921) found NiO to promote a laboratory model of photosynthesis from CO<sub>2</sub> or from formaldehyde, and conceivably it may have a similar function *in vivo* (Evans and Uri, 1951). However, the oxides of Fe, Co, U, and Be also catalyse this laboratory reaction. Ni resembles Cu in forming ammines, interesting complexes with NH<sub>3</sub>.

Manganese has been shown to promote the growth of many mammals and of protozoa (Hall, 1941). Deficiency causes perosis, or rarefaction of the bone, and "slipped tendon"; both are conditions which result also from deficiencies of vitamin B<sub>12</sub>, biotin and choline (pp. 315, 310, 317). Micromelia, a failure of growth specifically in the limb skeleton, also results and this resembles the effect of a deficiency of another of the B-vitamins, nicotinamide (Caskey and Norris, 1940). Mn is essential also for the special growth processes associated with reproduction, in both sexes in the mammals, and here the effects of a deficiency resemble those of vitamin E. The element is necessary also for the synthesis of vitamin C (Rudra, 1939), so that its relationships with the vitamins are probably more extensive than those of any other inorganic element. For plant growth 10<sup>-9</sup> is an adequate concentration (Heilbrunn, 1952).

Mn improves nitrogen fixation by leguminous plants and probably catalyses the synthesis of the special haem-protein, leghaemoglobin, which occurs in the nodule bacteria of these plants (Virtanen, 1947). It also promotes the synthesis of the magnesium porphyrin, chlorophyll (Heilbrunn, 1952). It speeds the assimilation of glutamic acid (Sexton, 1953, p. 267). It is a cofactor for carboxylase, which may explain its connexion with biotin, and why excess thiamine increases the requirement for Mn. Manganese is a cofactor also for a large number of other enzymes, phosphatases, phosphorylases and nearly all kinases (Long, 1961), arginase, dipeptidase, laccase, and carboxylase, and this is its main use as raw material. In the blood it is carried as "trans-manganin," a protein complex. Excess Mn is toxic, causing "lactation tetany" in cattle and a Parkinsonian type of tremor in Man; excess and deficiency, therefore, both seem to affect calcium metabolism.

Molybdenum promotes the growth of animals (Teresi, 1942) but excess, as in the "teart" grass of parts of Somerset, is deleterious and reduces the milk yield of cows (Fergusson *et al.*, 1938). Molybdates are powerful precipitants of protein, and may inhibit the copper enzymes (Comar *et al.*, 1949) for this, if not for more specific reasons. They also complex with phosphate, and cause the hydrolysis of its organic compounds (Lutwak and Sacks, 1953). The

importance of a correct, and in this case very low, concentration is again emphasized. For plants the optimum is  $0.1\ \mu\text{g}$  per litre of fluid absorbed. Mo promotes the reduction of nitrate to organic form by plants (Virtanen, l.c.). As fabric material it is a cofactor for certain flavoprotein enzymes, including that for nitrate reduction (Kit, 1960). There is an interesting antagonism between Mo and Cu (Miller and Engel, 1960; Calder, 1959).

Vanadium is a powerful growth promotor for plants (Arnon and Wessel, 1953), and the relative abundance of such a rare element in egg-yolk and in some tissues (Daniels and Hewston, 1942) may indicate a similar role in animals. It accelerates cell division in *Chilomonas* at concentrations lower than  $10^{-5}$  (Bowen, 1940). Like most of the preceding metals of this group it is potentially an oxidation-reduction agent, and also like them it is deleterious above its normal low concentration (Arnon and Wessel, l.c.). As a raw material it is required for the blood chromogen of many tunicates, in the plasma or in the corpuscles. Here a respiratory function would be expected but is far from certain (Webb, 1939); the chromogen can only function as a reversible redox agent at a low  $\text{O}_2$  tension and a low pH, outside the average physiological ranges. However, there is a high concentration of  $\text{H}_2\text{SO}_4$  in some tunicate blood corpuscles. A vanadium derivative of pyridoxal phosphate is coenzyme for cysteine dehydrogenase (Berzel *et al.*, 1958), and other metabolic functions of Va have been suggested.

Of the remaining metals aluminium (Group III) is toxic in high concentration; it depresses phosphate absorption and so may cause a type of rickets. However, it may be normal fabric material as a constituent of succinic oxidase (Horecker, 1939), and a basic aluminium succinate has been isolated from the tree *Orites* (Read, 1935, p. 316). There is as much as 40 mg/kg in onions and considerable amounts in some tumours. Gallium, of the same group, stimulates the growth of duckweed (Steinberg, 1941) and of *Aspergillus* (Fearon, 1949, p. 26). Vitamin D causes it to be deposited in bone, as it does calcium (Dudley and Friedman, 1952). Thallium inhibits growth and causes the hair to fall out. It is, therefore, used as a depilatory to stop the spread of ringworm in children, but curiously becomes too toxic for adults: in general adults have more resistance than children to toxic agents. In young rats it depresses the growth of various parts of the skull disproportionately.

In low concentration, selenium (Group VI) promotes the growth of plants (Williams, 1937; Nicol, 1942) and some plants accumulate considerable quantities (Haslett, 1952). In higher concentration it is poisonous, particularly for animals (Trelease and Martin, 1936). In parts of Dakota excess causes a disease known as blind staggers, or alkali disease in cattle, accompanied by the shedding of hair and hooves. It is used as a systemic insecticide, that is one which works by being fed to the insect's food plant (Martin, 1949).

Selenium resembles sulphur in its properties and is readily incorporated as a S analogue into amino acids and so into proteins (Cowic and Cohen, 1957). This may be the main danger of excess, but selenomethionine in place of

methionine seems to have little deleterious effect on the properties of  $\beta$ -galactosidase (Cohen, 1959). It may be a normal constituent of cytochrome *c*. In the form of selenite, Se specifically poisons succinic dehydrogenase, but  $\text{SeO}_2$  is not so poisonous as  $\text{SO}_2$  and the sulphites, and it may be in some such redox form that Se is required as a cofactor for the respiratory coenzyme Q, ubiquinone (Green *et al.*, 1961). Arsenic counteracts the toxicity of Se to some extent (Alexander, 1948)—one of the more impressive examples of two blacks making a white! Tellurium resembles selenium in most of its properties, but is less poisonous, because of its higher molecular weight and consequent lower reactivity.

Most other metals inhibit growth except in very low concentration. Lead (Bell *et al.*, 1930) and beryllium (Needham, 1941) are remarkably powerful inhibitors at concentrations well below the lethal level. Beryllium salts arrest cell division (Chèvremont and Firket, 1949), and cause anaemia of the macrocytic type (Stockinger *et al.*, 1951). Insoluble Be compounds may cause cancer (Hoagland, 1950). It has been known for over 40 years that Be causes a form of rickets (Kay *et al.*, 1931) and it now appears that this results from an inhibition of phosphate-mobilizing enzymes (Dixon and Perkins, 1956) rather than from simple competition with its analogue, Ca, for deposition in the skeleton. It probably *is* acting as a competitive analogue, but of Mg rather than Ca, though the latter is an effective cofactor for some of the P-mobilizing enzymes. In low concentrations Be can probably deputize for Mg in physiological functions, and stimulates the growth of plants (Hoagland *et al.*, 1952). Mn also is a very good cofactor for phosphokinases, and it is interesting that as long ago as 1888 Sestini found that Be could deputize for Mn as a promotor of plant growth. It also inhibits the effect of oestradiol on water intake and growth (Roberts and Szego, 1953). As already indicated (p. 179) it is a potent protein precipitant.

Arsenic and antimony, as arsenates and antimonates, competitively inhibit the action of phosphates, causing the breakdown of the hexose phosphate link, and blocking glycolysis. The effect on the growth of microorganisms is dramatic. In mammals amino acid uptake by the cell particularly is affected (pp. 184, 188).

Boron is probably the most important of the minor non-metals, and is a plant growth factor (p. 269) in very low concentration (Brenchley, 1927). It may be a cofactor (Rosenberg, 1946) in the synthesis of inositol (p. 296), one of the vitamins. It is present in relatively high concentration in some marine animals but it has not as yet been shown to be necessary for their growth (Hove *et al.*, 1939). It is very toxic in quantity (Trauter and Messer, 1953). It forms a host of very interesting organic compounds (Gerard, 1961).

Silicon is extensively used as a skeletal material by both animals and plants (p. 162), and this may be its only significance in growth. It constitutes 0·7 per cent of the weight of timothy grass and is the most abundant inorganic element in the plant, apart from K. As much as 77 per cent of the ash of feathers

is Si and here it is normally associated with sterols (Fearon, 1949, p. 28). There may be as much as 1 to 2 mg per cent in the serum of herbivores. As silicate it is a good example of an intractable material successfully exploited by living organisms because of its practical value. As an analogue of organic carbon it has trivial importance since it does not, in nature, form large stable polymer molecules.

Iodine, a vital growth factor for vertebrates, as the essential constituent of thyroxin (p. 373), also stimulates the growth of plants (Powers, 1939). Excess iodine may cause sterility (Adler, 1914) and excess thyroxin itself is deleterious (p. 375). Both I and Br are required as constituents of spongian and of anthozoan skeletons in the forms of diiodo- and dibromo-tyrosine. Diiodotyrosine is a structural intermediary in the construction of thyroxin. The tyrian purple pigment of *Murex* and other molluscs is dibromoindigo, a tryptophan derivative. The Br<sup>-</sup> anion can replace Cl<sup>-</sup> to some extent, just as for certain purposes Sr can replace Ca, Li can deputize for Na, Be for Mg, and Se for S. In all cases the extent of this is limited and emphasizes the high specificity of the requirement for each element. Cl is important mainly as the chloride anion, associated with Na, K, Ca, and Mg, though about 15 to 20 per cent is in organic combination. Cl<sup>-</sup> is a cofactor for salivary amylase, and NaCl helps to retain the body's water. Fluorine is essential in low concentration for the growth of bone and teeth, but excess causes brittleness, with mottling of the enamel. Concentrations as low as 0·02 per cent inhibit growth in some other tissues. Fluorides depress milk yield; they are well known as inhibitors of certain steps in the glycolytic sequence (p. 258), and of other reactions.

Fluorine, therefore, is typical of a large number of inorganic elements, essential for growth in very low concentration and very toxic in higher concentration. Calcium and silicon are almost unique in the range of their concentrations which are tolerated, but this is probably due to their relative insolubility. Phosphate also probably would not be tolerated in high concentration except in such insoluble forms as the hydroxyapatite of bone. Most elements are used for a very precise and restricted purpose and this demands a very restricted range of concentration. Another general feature which emerges is the large number of elements which have been exploited and have become essential as constituents of the fabric or as agents for growth. The many highly specific functions necessarily demand a wide range of specific agents. It is very rare that one element can deputize for another, and probably never fully and permanently. Each is unique in the sense that there can be only one which is best for each purpose. At the same time most of the elements interact with one or more others in the body, either increasing or decreasing their actions, so that collectively they form part of an integrated system, completed by the organic components.

### 17.3. Organic Constituents of the Diet

From the preceding section it is clear that inorganic elements play an important part in the growth of animals, but it is the organic constituents of the

diet which build most of the body's fabric and supply virtually all the energy required for this. It is profitable to consider the organic constituents collectively before turning to the specific functions of the individual classes of material, since they are interdependent in a number of ways. The ascending order of treatment might seem to require that the individual classes should be considered first, but the food is first encountered as a complex mixture and this justifies the present procedure. As in the case of the energy supply (Chapter 16), the quantitative and qualitative aspects of dietetics should be distinguished.

### 17.3.1. The Quantity of Food

In the growth of bacteria, and of protozoa (Bond, 1931; Richards, 1941) and other animals, a linear relationship between growth rate and available food, or food consumed, has been demonstrated. This simple relationship is not maintained indefinitely, however, and rate tends to a ceiling value (Hinshelwood, 1944). The growth machinery presumably is now working to full capacity, although in theory it also should be able to expand with the mass of the body. The limitation on rate, therefore, is presumably due to restrictive controls acting at a higher level (p. 360). This restriction probably decreases with any decrease in intake so that after partial starvation animals make better use of any food offered than do those which are well fed (Robertson, 1923, pp. 225, 241; Brown, 1957; Wilson and Osbourn, 1960). Of course the position here is somewhat complicated by the fact that there is a higher food requirement for mere maintenance (p. 253) in the more bulky, well-fed animal. For this same reason the maintenance requirement of an adult human being is absolutely greater than that of a growing child and greater even than the total requirement of a young child for both maintenance and growth.

In higher organisms food intake itself is regulated, at the organismal level, by appetite; this ensures an optimal, as opposed to a maximal, consumption. There is an appetite-regulating centre in the hypothalamus (Kennedy, 1950; Mayer *et al.*, 1951) controlled, in part at least, by the level of glucose, and of glutamic acid derivatives, in the blood. It is possible to strain this mechanism by persistent overeating, prompted by skilful menus and cooking; the digestive mechanism responds to training in the same way as other physiological systems. As Shakespeare put it ". . . appetite had grown by what it fed on. . ." (*Hamlet*, I, 2). This is one cause of accelerated growth in the young, and of fat deposition in the adult. In other individuals, however, this may be due to inherent imperfections in the mechanism. Normally there is an effective negative feedback mechanism, and young animals neither eat to excess nor grow too rapidly. Sinclair (1955) has questioned the virtue of maximal feeding and of a maximal growth rate both on this and on other grounds. Rapid growth due to this cause is correlated, in Suctoria (Rudzinska, 1952), Rotifera (Lancing, 1947), Cladocera (Ingle, 1933, 1937), and other animals with earlier maturity and a shorter life span. There appears to have been an acceleration of growth and earlier maturity, in European children, associated with the improved diets of the

last fifty or more years (Morant, 1950), and although no proportional shortening of life span has been detected, this might be due partly to the masking of such a tendency by improved health services.

It is not easy to determine the optimal growth rate. The best ethical criterion would seem to be the production of the maximal span of healthy life—life to our years and not merely years to our life—as the gerontologists put it. Productive gait would be a reasonable compensation for some degree of brevity. In any case it is very possible that “the joyfulness of man prolongeth his days” (*Ecclesiasticus*). A slowly growing, underfed animal reaches a normal maximal size in a longer time than normal but this does not prove that there is virtue in growing slowly; it may merely illustrate the ability of the growth mechanism to cope with difficulties. We may find that it is not so much total age-span as rate of growth and of living which are most valuable, in human beings at least. Lát *et al.* (1960) found that rats, rapidly growing through good feeding were more active and better able to learn. A high dietary level helps to counteract the inhibitory effect of carcinogens (p. 107) and of radiations on growth (Elson, 1949).

Another possible criterion, particularly in animal husbandry, is the growth rate giving maximal efficiency in the use of fuel and material (p. 252). The assimilation of fat is better on a periodic rather than on a continuous pattern of intake (Medes, 1952), and Kopec (1938) found that one fast-day a week resulted in a greater final size in rats. On the other hand the interval between meals passes through an optimum; if trout fry are fed at excessively long intervals they eat more but grow less (Brown, 1946). Digestion is probably less efficient.

Another criterion for optimal growth-rate might be that rate which gives a maximal or at least an optimal final size. In fact, final size appears to be relatively constant and to have been very little changed by the acceleration of growth in recent generations of European children (Morant, *l.c.*), and the most plausible algebraical definitions of growth (p. 13) recognize a final size which is independent of the rate at which it is achieved. Even so, in practice it is found that some environmental variables do affect final size and again the optimum may be difficult to determine. However, with the intuitive knowledge that subnormality is far more easily caused than serious excess, opinion would place the optimum near the maximum: “a good big ‘un is always better than a good little ‘un.”

A maximal final size in rats results from a moderate degree of underfeeding as measured by appetite and by growth-rate (McCay *et al.*, 1943; Kopec, 1938). At both higher and lower nutritional levels the final size is smaller, rather by analogy with the maximal altitude of a missile, which is achieved at an intermediate angle of projection. This may prove significant in the explanation of the curious fact that the hydroid *Campanularia* casts its hydranths most frequently if either over- or under fed (Crowell, 1957). Breeders of livestock in general would probably agree that a slight degree of underfeeding during the main growth period is optimal both on this criterion of maximal size, and on that of economy and efficiency.

In explanation of this it may be suggested that the processes of differentiation and maturation which normally set the term to growth are less sensitive than the latter to a shortage of food. Consequently starved tadpoles metamorphose as dwarfs (Krizenecky, 1914), and plants usually flower, however stunted in growth. In animals, also, maturation and the reproductive function are relatively resistant to underfeeding. On the other hand, differentiation may be more sensitive than growth to an enhanced food intake and subject to no comparable ceiling rate. Moreover, the need to catabolize the excess food may itself stimulate some aspects of differentiation. Consequently size is maximal under intermediate conditions. Not all cases fit this picture, however. Young mice suckled by rats not only grow more rapidly than under normal conditions but also reach a greater final size (Parkes, 1928). Further, it seems that this is due to nothing more than the greater quantity of milk from their foster parent.

Within limits growth may be halted by starvation and resumed upon refeeding (Wilson and Osbourn, 1960), and this is also true of regenerating limbs, for instance in the shore crab, *Carcinus*. Starvation retards the growth of organs differentially (p. 29) so that insects have relatively long wings when underfed (Child, 1939).

The general conclusion is that there is an inherent growth mechanism which requires a certain optimal level of nutrition but that it can adjust to a considerable range of levels without giving very marked symptoms of abnormality. In consequence it is not easy to find the optimal level, that which puts least unnecessary strain on the animal's metabolism. Most normal animals probably have their inherent appetite-regulating mechanisms and eat just enough to grow at the biologically optimal rate. Man's idea of the optimal rate may be influenced by considerations which cut no ice with Natural Selection.

### 17.3.2. The Quality of Food

“A man's own observation, what he finds good of and what he finds hurt of, is the best physic to preserve health.”

BACON: *Of Regimen of Health.*

The nature of the diet is a more critical consideration for the growth of heterotrophic organisms than for that of autotrophes, which necessitates only a relatively limited number of simple raw materials: carbon dioxide, the nitrates, sulphates and phosphates of the appropriate alkali metals, together with small amounts of other metals and non-metals, and adequate water; from these they synthesize all their requirements. A typical animal requires for growth and maintenance the familiar four main categories of organic material: proteins, fats, carbohydrates and vitamins, in addition to water and salts. No absolute demand for nucleic acids has been recorded and they are probably synthesized in adequate amounts in the body. The fundamental qualitative requirements are very similar in all animals, and also their relative proportions. The complexity

of the mechanism controlled by qualitative requirements is illustrated by the experiments of Reynolds (1942) on young *Tribolium* (p. 399). These mealworm larvae actually grew more quickly on the qualitatively deficient white flour than on whole meal, but their mortality was higher. The quality of the diet probably affects the quality of the animal. Brody (1945, p. 741) quotes Brillat-Savarin: "Tell me what you eat and I will tell you what you are. The destiny of a people depends on the nature of its diet," and there have been indications that this was a very perspicacious intuition.

The importance of a qualitatively balanced diet is heightened by the need for a more or less simultaneous presentation of all dietary constituents, in correct proportions (Geiger, 1948). Thus adequate amounts of the vitamins increase the efficiency of utilization of other components, even from the initial stage of digestion (Sinclair, 1948). This may permit healthy growth on a lower total consumption (Brody, l.c., p. 153; Bauer, 1952). Protein is most valuable if fed along with carbohydrate (Cuthbertson and Munro, 1939): dogs can digest a mixture of starch and raw egg, but neither alone. An unbalanced diet usually exerts a higher specific dynamic action (p. 254) so that more of it is immediately catabolized. Further, since the normal relationships among such components as amino acids are not always synergistic (p. 289) but often competitive (Roblin, 1946), an imbalance between them may necessitate the destruction of any which are in excess. In our own diets we are so accustomed to something reasonably near simultaneous presentation that we may under-estimate the temporal hazard for some wild animals and perhaps even more for autotrophes.

The quality appetite or "palate" of animals probably enables them to select the correct quality of their food as wisely as the quantity. The craving for water naturally is the most demanding, but deficiencies of fat, sugar (in diabetes), protein (in pregnant females), salt (African wild animals, and sufferers from adrenal-cortex deficiency) and calcium (parathyroidectomized mammals and pregnant females) cause appropriate cravings. Deficiency of thiamine prevents the normal conversion of carbohydrate to fat (p. 310) and causes a craving for fat. Richter and Eckert (1938) and others have found that rats, offered a complete range of pure, or relatively pure, food constituents, show a remarkably accurate selection of a balanced diet, the total quantity being actually lower than on the stock laboratory rations (Thorne, 1949) which, therefore, must be rather less economically used. Chicks select 18 per cent protein, 56 per cent carbohydrate, 4·7 per cent fat, 7·1 per cent salts and 3·5 per cent fibre, or roughage (Brody, l.c., p. 18), which is quite similar to the ratios recommended for human beings on the basis of experience and experiment. Again the total amount is lower than when eating the less well-balanced, stock rations, but egg production is as good, so that selection permits an optimal, that is, a most economical intake.

In recent years there have been a number of serious doubts about the wisdom of quality choice (Tribe, 1952; Sinclair, 1948). Chicks select thiamine-containing foods when deficient in the vitamin, but there is no comparable

wisdom for riboflavin. Children appear surprisingly unwise in some of their choices, and insects are misled by the mere flavour of a normal, essential food added to worthless material (Trager, 1953). Children are guided by colour, texture, and so on, which may camouflage valueless foods. However, it must be remembered that flavours divorced from their normal nutrient matter are not usual in nature and that, as pointed out by Dr. F. A. L. Clowes, an already well-fed child is no subject for serious dietary experiments. Equally, starvation compulsively overrides the power of qualitative discrimination and animals can be poisoned rather easily in this state. Moreover, even under relatively normal conditions the palate, like the quantitative appetite, can become warped by bad habits. Many people prefer white bread to the more complete whole-meal loaf, though the evidence from the *Tribolium* larvae offers a possible rationale for this. Unconditioned children choose wisely (Harris, 1933). The choice of amino acids is said to be rather poor, but again, individual amino acids are rarely found free in nature. Some of them do have attractive tastes and no doubt assist choice: for instance threonine has a spicy odour (Phillips, 1954), methionine a flavour of pineapple and cysteine a beery odour, while glutamic acid is used as a flavouring in the orient.

Under natural conditions animals probably are rarely subjected to these complications in selection, and their choice must be reasonably sound, when based on the mixture of essential constituents present in natural foods. Pure, single components are rare as natural foods and it is not surprising if the ability to select particular ones isolated in the laboratory is not perfect. The preservation and processing of human food therefore has introduced a great nutritional danger since it may destroy differentially a few components in the staple foods, leaving them almost as palatable as when fresh, but possibly inferior in nutritive value. Moreover when the resulting deficiency does find expression in an appetite demand, this is quite likely to be for the same food—because in its natural state it would have supplied the components which are now lacking. The unbalance therefore is further exacerbated, since the components which are present increase the requirement for the others and a vicious circle is established. The solution of this as a commercial problem is formidable, even with the maximum of public spirit among food manufacturers. It is expensive either to prevent recognized losses or to make them good by specific supplements. The processed forms usually keep better than the fresh food and some, such as white bread, have the attraction of being more rapidly digested than the crude counterpart. On the other hand, some foods possibly lose more in "palate" than in nutritive value by processing, and this again may adversely affect the wisdom of quality choice.

In hot climates the human appetite for fats and proteins decreases relatively to that for carbohydrates. This is wise since fats conserve heat and proteins produce it through their specific dynamic action (p. 254), which is about ten times that of carbohydrate. The palate varies also with occupation: men doing sustained heavy manual work prefer the high-energy fuel, fat (p. 297). There

are age changes also: children enjoy carbohydrates most and fats least, though their protein requirement is a higher percentage of their total diet than is that of adults (*below*). The amount of the thyroid hormone in the body affects quality choice (Donhoffer and Vonotzky, 1947), so that this is probably sensitive to variations in metabolic activity and the consequent changes in nutritive requirements.

It therefore seems that the higher animals have considerable ability to choose a well-balanced diet from natural biological materials and to recognize those which contain individual factors particularly needed at the moment. The palate varies with changing requirements, depending on environment, activity and so on. It is probable that other animals have equal powers; for instance, *Hydra* is very sensitive to glutathione (Loomis and Lenhoff, 1956). Even amoebae select nutritive matter in preference to sand grains and some Foraminifera have possibly developed an artistic taste (Sandon, 1957) out of this ability!

### 17.3.3. Proteins

The structural and functional materials of the body, apart from skeleton, and water, are mainly protein. Food protein is therefore the most important single component of the diet. Body protein increases isoauxetically (J. Needham, 1931) with total body-weight, and is the best single measure of growth. The rate of weight-increase by nursing mammals of different species is closely proportional to, though of course not equal to, the protein content of the mother's milk (Hawk *et al.*, 1947, p. 204): the latter is exceptionally low in human beings, which grow unusually slowly (p. 11). Similarly, within any one species growth rate is linearly related to protein intake, over a considerable range of the latter (Almquist, 1951). Pigeon's "milk" from the crop of the parents, contains 19 per cent protein, a concentration which compares with that of the richest of mammalian milks. The growth of the squabs is correspondingly rapid (J. Needham, 1942, p. 75). The optimal protein content of food is 14–18 per cent, and in rats this also gives the maximal life-span (Slonaker, 1931). At this level there is minimal destruction or conversion to non-nitrogenous fuel material, as measured by the level of excretory nitrogen in the blood (Addis *et al.*, 1952). However, the percentage in the diet may be varied within wide limits without very serious effects on growth and health: thus on the one hand Eskimos traditionally ate a diet containing 40 per cent protein and on the other hand the Javanese one of 9 per cent. With a total intake of 3,000 calories per day, 18 per cent as protein amounts to something under 100 g, or a little over 1 g per kilogram of body weight in an adult man. Some experiments have indicated that much less than this is adequate. The requirement per kilogram declines steadily from 3.5 g in infancy—

Age (years)	1-3	3-5	5-15	15-17	17-21	21
Protein requirement (g/kg. day)	3.5	3.0	2.5	2.0	1.5	1.0

The different food proteins vary considerably in their value for growth. This depends mainly on their amino acid composition. The best are those with a wide variety of the "essential" amino acids which cannot be synthesized from suitable precursors by the animal itself. The essential amino acids are leucine, isoleucine, valine, methionine, threonine, arginine, histidine, lysine, phenylalanine, tryptophan; the non-essential acids are glycine, alanine, norleucine, serine, cysteine, aspartic acid, glutamic acid, proline, hydroxyproline and tyrosine.

On this basis, the nutritive value for animals, a distinction has been made between first- and second-class proteins; it is not surprising that in general this proves to be the distinction between animal and plant proteins. Plant proteins are commonly deficient in one or more of the essential acids, for instance zein, from maize, is deficient in lysine and tryptophan. However, some animal proteins, usually specialized ones, also may have serious deficiencies as food; thus gelatine from collagen (p. 164) lacks tryptophan and phenylalanine. In early experiments on rats (Osborne and Mendel, 1916) diets containing 8 per cent of edestin, from cotton-seed, casein from milk and lactalbumin from milk gave average gains of 50, 71, and 77 grammes respectively, in body weight, in equivalent times. Thomas obtained the following relative values for the nitrogen-sparing action of different proteins (Robertson, 1923, p. 225): Beef 104, milk 100, fish 95, rice 88, cauliflower 84, potatoes 79, yeast 71, casein 70, spinach 64, peas 56, wheat 40, cornflour 30. Milk proteins and egg proteins rather naturally are among the best-balanced of animal proteins since they are normal provision for the young. Even herbivores seem to prefer animal protein if available. Carnivores in general live longer than herbivores, but perhaps there is only one degree of freedom here!

A mixture of different proteins, collectively providing all the essential amino acids in the right proportions, may promote growth as well as a single balanced protein, and wheat is a good food because gliadin and glutelin, its two main proteins, synergize well; the former is poor, and the latter very rich, in lysine. Milk, meat and other animal materials synergize well with plant seed proteins, which are generally poor in lysine. Breakfast cereal with milk, or ham sandwiches, therefore, constitute first approximations to a balanced diet.

It is clear that the food proteins are used mainly as their free amino acids (p. 203). There is evidence that particular food proteins go differentially into the various body proteins; for instance egg proteins go extensively into plasma proteins and beef proteins into haemoglobin. This must again be due to the degree of similarity in their respective amino acid compositions. It would be anticipated that the ideal proteins are the homologous proteins of the same species and there is evidence in favour of this. It has been claimed further (Fischer, 1946) that for cells *in vitro* homologous proteins promote growth better than a mixture of heterologous proteins giving the same ratios of the various amino acids. This imputes an importance to properties other than gross amino acid composition, most probably to the amino acid sequence. If so, the

possibility is again raised that suitable amino acid sequences for building may be utilized without complete hydrolysis (p. 203). There is some indication (Fisher, 1954) that food proteins may be absorbed from the gut, and therefore perhaps also utilized, without complete hydrolysis; conceivably this depends on the suitability of their amino acid sequences for building the host's proteins. The question is considered further in the next section.

The optimal protein requirement in the diet cannot be considered in isolation from those for carbohydrates and fats. A balanced diet for adult Europeans contains protein, fat and carbohydrate in the approximate ratios 1:1:4 by weight but the absolute amounts also must be near the optimal values of 100:100:400 grammes. This provides adequate energy and fabric. If the three totals are reduced proportionately then nitrogen intake eventually falls below nitrogen excretion due to wear and tear in the body (p. 191), and this cannot be remedied by increasing carbohydrate and fat adequately to make good the caloric deficiency. If protein is maintained at 100 grammes or even increased, while carbohydrate and fat are diminished substantially, then again nitrogen imbalance results because considerable protein is then deaminated as a source of energy, to replace the deficient fuels. Man therefore never achieves nitrogen balance on a diet of protein alone, though Eskimos classically did so on virtually fat and protein alone. The normal person surfeits of protein before reaching equilibrium. Given the correct absolute amount of carbohydrate and fat but excess protein, again the excess is deaminated and nitrogen output increases proportionately to protein intake. The  $\alpha$ -keto derivatives of the deaminated amino acids are converted to fat or carbohydrate, depending on whether they are ketogenic or glycogenic. They are converted to various intermediaries which feed into the glycolytic and TCA systems (p. 258), or are stored as polysaccharide and fat.

Another factor which prevents man from establishing equilibrium on a diet of pure protein is its high specific dynamic action (p. 254) compared with that of fats and carbohydrates. When a fasting animal takes a meal of protein, catabolism and heat production increase as much as 30 per cent. The stimulation of metabolism is due partly to the deamination process but in any case it results in wastage of much of the energy supplied as protein. Moreover, the resulting strain on excretory metabolism is deleterious. A balanced diet is essential for both growth and health (maintenance).

#### **17.3.3.1. Peptides**

The hydrolytic degradation products of proteins, that is proteoses, peptones and peptides, often support growth better than do native proteins, and this is to be expected since hydrolysis is a preliminary to the utilization of protein. This is the rationale for invalid foods based on peptones; free amino acids would be even more readily assimilated, but would be less satisfactory commercially. Peptones have not been marketed because these intermediaries support growth more vigorously than free amino acids (p. 285), though they may do so in some

cases (p. 203), and some microorganisms appear to have a specific requirement for peptides as such (Simmonds and Fruton, 1949*a, b*; Waelsch, 1952). However, in some instances where dipeptides were found more effective than free amino acids (Simmonds and Fruton, 1951; Kihara, 1952; Peters *et al.*, 1953) the reason was simply that the peptide bond protected the constituent amino acids from decarboxylation and other degradative changes, and the conclusion reached on p. 203 still stands.

The limited number of definitive oligopeptides in our body, or produced by other organisms, constitute a special category. The very widely distributed tripeptide glutathione (GSH), glutamyl- $\gamma$ -cysteinyl glycine, has outstanding growth-promoting properties (Colowick *et al.*, 1954; Bartlett and Stevenson, 1954; Kidwell *et al.*, 1955). It is very abundant in rapidly growing young embryos (Brachet, 1950), and more abundant in rabbits which grow to a large adult size than in small races (J. Needham, 1942). It is more concentrated in growing and in regenerating liver than in non-growing liver (Bartlett *et al.*, 1956), and the concentration here is increased by the action of the anterior pituitary growth hormone, APGH. Like some other —SH compounds, GSH promotes mitosis (Mazia, 1956) and diminishes the inhibitory action on this process of iodoacetate (Rapkine, 1931), nitrogen mustards and other oxidizing agents. Here, as in general metabolism, the reducing action of GSH is its main property (Bricas and Fromageot, 1953), and cysteine, whether free or in protein combination, often has equal value. However, in glyceraldehyde-3-P dehydrogenase, it would appear that the entire GSH molecule, bound to protein, constitutes the active prosthetic group (Krimsky and Racker, 1952).

It is less certain that the other two components, glycine and glutamic acid, contribute to the growth-promoting properties of GSH, though glutamic acid in isolation has outstanding importance (p. 289). Transpeptidation by GSH, which depends on the glutamic acid component, does not appear to be important for natural protein synthesis (p. 194), but it may be significant that all three constituent amino acids readily form peptide bonds with a variety of substances (p. 292). Admittedly some of these are detoxication reactions (Carter and Thompson, 1953), but there is evidence that GSH is involved in the transfer of amino acids from the soluble RNA to peptide linkage (Nathans, 1960).

Some of the pituitary hormones are oligopeptides, and there may be other oligopeptides in the body fluids (p. 203). Antibiotics (p. 404) are often oligopeptides, for instance gramicidin and bacitracin, and these are usually inhibitory to the growth of other organisms. This action depends on such properties as the inclusion of one unnatural amino acid, usually the D-isomer of the natural amino acid. This kind of abnormality may explain why some laboratory oligopeptides have been found to inhibit growth strongly (Dunn and Dittmar, 1950). Some of the oligopeptides have a cyclic structure, which no doubt stabilizes the molecule.

### 17.3.3.2. Amino Acids

The importance of providing all the essential a.a.s simultaneously if protein synthesis and growth are to be maximal (Geiger, 1950; Gale, 1953) is probably the best demonstration that this is their function. They are used individually for other purposes, for instance arginine for urea synthesis, and it has been suggested that this explains some instances where simultaneity of administration appears not to be essential. Of course, simultaneity does not imply equality of amount: even in the body's proteins collectively the proportions of the various a.a.s vary considerably. The use of individual a.a.s for other purposes therefore may or may not increase the disproportion in requirements. One of these purposes is the synthesis of the non-essential a.a.s, but these also are partly at least supplied in the diet and partly synthesized from precursors other than a.a.s. Consequently adults rarely suffer from a deficiency of glycine, although it is one of the most abundant a.a.s in proteins and is also required in quantity for the syntheses of purines, porphyrins, creatine, choline and other metabolites. It seems probable that the proportions of the various a.a.s in the proteins collectively may be much the same in all animals so that if a.a.s were only required for protein synthesis carnivores should have an already balanced intake. As it is, however, probably all animals need to consume an excess of protein food in order to obtain enough of the a.a.s required for other purposes; there is therefore some catabolism of the excess of the other a.a.s even when the non-nitrogenous components of the diet are providing enough energy.

It is noteworthy that the list of essential amino acids (p. 285), although generally similar in diverse animals, does vary somewhat. Some bacteriophages demand even glutamic acid, aspartic acid and proline (Cohen, 1949); glutamic acid is demanded also by certain bacteria (Wooley and Hutchings, 1940; Feeney and Strong, 1942). Like the vitamins (p. 303) these are probably demanded because adequately provided in the normal diet. Demands also change during the life cycle. The young animal may be unable to synthesize even glycine rapidly enough for maximal growth, while on the other hand in the adult even histidine and lysine may become virtually dispensable in the diet. These last have been called by Gudernatsch (1934) the growth a.a.s and certain others, such as leucine, which are still demanded by the adult, were termed maintenance acids.

His third category, the differentiation a.a.s, phenylalanine, tyrosine and tryptophan, also has some justification: they are used for the synthesis of insoluble proteins, melanins and other stable, specialized materials (pp. 173, 245). They are therefore less important for early growth, and the average a.a. composition of the body proteins itself changes during development (Gustavson and Hjelte, 1951). Both essential and non-essential a.a.s are included in each group of Gudernatsch. The general validity of the three groups has been upheld by Wilson (1942-5).

Certain essential a.a.s such as lysine, have proved non-essential *in vitro* (Willmer, 1958), but conceivably they are provided by the embryo extract or

by other protein materials of the medium. Reciprocally there is a demand for cysteine, which *in vivo* is adequately synthesized from methionine. This may imply that there is a systemic site for this synthesis.

A factor of some importance is the interaction between a.a.s. It has been seen (p. 184) that they influence the uptake of each other into the cell, and probably the subsequent incorporation into proteins (p. 189). The interactions may be positive or negative, promoting or inhibiting protein synthesis. By forming small peptides outside the cell some may depress the genuine protein synthesis inside. However, in a balanced mixture of a.a.s, presented simultaneously, the various deleterious bilateral interactions are controlled at a minimal level and synthesis is maximal. The interactions range from relatively non-specific to highly specific; of the latter the most important type to emphasize is the competition between analogous a.a.s (Roblin, 1946), for instance between—

$\alpha$ -alanine	and glycine
serine	and threonine
glutamic acid	and aspartic acid
asparagine	and $\beta$ -alanine
lysine	and arginine
leucine	and isoleucine, valine
isoleucine, valine	and norvaline, norleucine, serine, phenylalanine
methionine	and nor-leucine, glycine, arginine

Competing a.a.s often increase the requirement for each other, since the outcome of competition depends on the relative concentrations of the contestants. In other cases, however, an a.a. may appear to increase the requirement for another by actually improving its utilization, and so its subsequent depletion. This kind of action is shown also by vitamins (p. 306) and other growth agents.

There is not space to consider in detail the special functions in growth of all the a.a.s (Haurowitz, 1950; Baldwin, 1953; Neurath and Bailey, 1953), that is to say their functions other than as raw materials for protein synthesis. It is evident, of course, from the variety of side-chains among the common a.a.s that each has its own special role in the subsequent work functions of the body, if not in growth itself. Thus methionine,  $\text{CH}_3 \cdot \text{S} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$ , is a methyl donor, of its  $\omega\text{-CH}_3$  group, for the synthesis of choline and other heteromethylated compounds. It is also a donor of its sulphur atom (p. 268) for other syntheses. A number of the other a.a.s similarly have quite a range of functions (p. 245), but glutamic acid is outstanding for the multiplicity and importance of its activities in growth itself, and this a.a. merits detailed consideration.

### 17.3.3.3. Glutamic Acid (GA)

This dicarboxylic amino acid,  $\text{HOOC} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}(\text{NH}_2) \cdot \text{COOH}$  is outstanding rather than merely typical, in the variety and extent of its metabolic

activities, many of which are directly or indirectly concerned with growth. In plants some, but by no means all, of its functions are performed by its C<sub>4</sub> analogue, aspartic acid, which also forms an  $\omega$ -amide, asparagine, corresponding to glutamine (GN), H<sub>2</sub>N·OC·CH<sub>2</sub>·CH<sub>2</sub>·CH(NH<sub>2</sub>)·COOH. So much of the activity of GA depends on its ability to form this  $\omega$ -amide that it is perhaps reasonable to consider the two together, though there are instances where a requirement has been found for GN but not for GA. The subject has been reviewed by Archibald (1945), Braunstein (1947), Zamecnik and Aub (1950), Waelsch (1952), and Meister (1956). All illustrate the manifold functions of the two substances.

As a building material GA is the most abundant amino acid in many proteins, particularly those of young animals and of seeds. In adults it is common in all except some specialized proteins. GN also, is abundant in seed proteins. Not uncommonly there are polyglutamic sequences in proteins, and free GA-homopeptides also are known (Dekker *et al.*, 1949). By laboratory methods, also, it is readily induced to form such polymers. GA is the most abundant amino acid in the anterior pituitary growth hormone (p. 365). It is a component of the tripeptide GSH, already considered, and this indeed could be regarded as a substituted glutamide, since the linkage with cysteinyl glycine is via the  $\gamma$ -carbonyl group. It is also a component of the B-vitamin folic acid, or pteroylglutamic acid (p. 314), and almost certainly of the peptide strepogenin, sometimes regarded as a B-vitamin. Polyglutamic derivatives of folic acid are common. In the laboratory it has been found to form polyglutamic compounds with another B-vitamin, pantothenic acid (King *et al.*, 1948). The capsule around some bacteria contains large amounts of a polymer of the unnatural, D-isomer of GA.

It is clear that the incorporation of GA into various compounds constitutes a significant fraction of biosynthesis, adequate to explain a widespread demand for GA or GN as a growth factor by microorganisms (McIlwain *et al.*, 1939; Wooley and Hutchings, 1940; Pollack and Linder, 1942, 1943; Hendlin, 1949), and its demonstrable importance for the growth and maintenance of almost all cells (Meister, 1956). In fact Gale (1953) has used the rate of incorporation of GA into Gram-positive bacteria as a measure of their rate of protein synthesis and this is consistent with the results of others (McIlwain *et al.*, 1948). The bacteriostatic, growth-inhibitory, effect of penicillin on Gram-positive bacteria may depend largely on its ability to inhibit GA-uptake by the cell (Gale and Taylor, 1947); significant amounts of GA are found only in penicillin-resistant streptococci, not in sensitive strains (Capper and Heatherman, 1950). This amino acid therefore is of major importance as fabric material.

If it were used only as building material the percentage of GA in the body should not vary much during the growth cycle. In fact, however, it is maximal when growth is maximal, and this seems good evidence that it is also an "agent" in the growth process. As a component of folic acid (p. 314), strepogenin, and GSH it would be important enough, but its actions seem to extend beyond this.

In the case of the sea-urchin an ability to synthesize GN from GA appears suddenly at fertilization (Waelsch, 1952), and during the cleavage both of this (Gustafson and Hjelte, 1951) and of the Amphibian egg (Kutsky *et al.*, 1953) it increases in amount more than any other amino acid. The relatively high GA-content in the proteins of young animals and a high content already in the egg-yolk proteins have the same significance. After starvation yeast incorporates nitrogen almost entirely into GA (Kutsky *et al.*, 1953). It is abundant in the lymphopoietic tissues, spleen and thymus (Krebs *et al.*, 1949) and GN increases during infection by some of the viruses. There is a high rate of GA turnover in tumour tissue. GN accumulates in muscle when growth is inhibited (Waelsch, 1952) and is depleted when growth is rapid (Gustafson and Hjelte, 1951); hypophysectomy also causes accumulation. This seems clear evidence of a function as growth agent, or as part of such an agent, and not simply as a raw material. In synthesizing polypeptides by laboratory methods, Fox *et al.* (1959) found glutamic and aspartic acids of unique importance. They were required in larger amounts than the other amino acids, and promoted the heteropolymerization of all.

GA has been shown to stimulate mitosis (Bullough, 1952), and it is a cofactor of the dicarboxylic plant wound hormone, traumatic acid (English *et al.*, 1939). It also promotes the uptake of other amino acids by the tissues of metazoa (Christensen *et al.*, 1948) and by microorganisms (Gale, 1953). In the latter, GA by itself is taken up very rapidly, and maximal protein synthesis is then obtained on presenting other amino acids: simultaneous presentation is not essential in this case, apparently. GA assists the absorption of iron by forming a soluble glutamate (Carter and Thompson, 1953) and it also promotes the uptake of the K<sup>+</sup> ion (Terner *et al.*, 1950), which usually accompanies growth (Fenn, 1940; Richards, 1938). This effect on the movement of K<sup>+</sup> may operate also in nerve conduction (Hodgkin, 1951) and it may be significant that GA is released in active electric organs (Fatt and Woodin, 1953); this is further evidence of the resemblance between growth and the work functions. Moreover, in nerve tissue, particularly, the recovery phase following work is associated with much protein synthesis (Hydén, 1943; Eccles, 1953). GA appears to be concerned with nerve conduction in other ways (Weil-Malherbe, 1952) and is highly concentrated in the brain (Krebs *et al.*, 1949): it affects the biosynthesis of acetylcholine (Augustinssen, 1952; Nachmansohn and Machado, 1943) and of other neurotropic amines (Weil-Malherbe, l.c.); it can also serve as fuel, for nerve (McIlwain, 1955; Price *et al.*, 1943) and other tissues (Barron and Goldinger, 1941). In some of these roles it is largely controlled by insulin but it is not yet clear how far all depend on a single property of GA (Bach and Holmes, 1937; Harris *et al.*, 1938; Mayer-Gross and Walker, 1949; Ginsberg and Roberts, 1951).

As already indicated, many of the properties of GA are due to peptidation activity, using its  $\gamma$ -carbonyl group. It is linked thus not only in GSH but also in bacterial capsule proteins and in other natural and laboratory peptides. In

PGA (p. 314) and probably also in normal proteins, however, it is  $\alpha$ -linked, in the usual way, and this is a main reason (p. 194) for scepticism about the Hanes transpeptidation-mechanism (Hanes *et al.*, 1950) as a step in normal protein synthesis. However  $\gamma$ -transamidation and transpeptidation (Waelsch, 1952) are so readily effected that the latter should still be regarded as a possible normal intermediary step. The bond is used also in the formation of carbamyl glutamate, in the process of urea synthesis, and in connexion with certain detoxication syntheses. GA also promotes detoxication syntheses by other amino acids, as in the reaction between glycine and benzoic acid to form hippuric acid, effectively a peptide condensation, and this is further evidence in favour of the wider transpeptidation function just considered. No doubt it is significant (*see* Carter and Thompson, 1953, p. 209) that the remaining amino acid of GSH, cysteine, also performs detoxication syntheses, since this gives some rationale to that particular tripeptide association (p. 287).

GN and GA are also able to supply the  $\text{NH}_2$  groups required for purine synthesis (*see* Baldwin, 1953, p. 327), and GN can also aminate glucose (Kalckar and Klenow, 1954) in the synthesis of chitin (p. 235). The  $\gamma\text{-NH}_2$  group is believed (Meister, *l.c.*) to assist in the transport of the glutamyl radical itself across the cell membrane, since GN is the more readily absorbed (Archibald, 1945). When the uptake of other amino acids is found to be accelerated by GA, it may form this  $\gamma$ -peptide link with them.

A related property of GA which also is of importance in growth and biosynthesis is its transaminating ability. Here its  $\alpha\text{-NH}_2$  group is transferred to the  $\alpha$ -position of the precursor of almost any of the other common amino acids, except lysine and threonine. Since GA, alone of the amino acids, can be formed by direct amination of its precursor,  $\alpha$ -ketoglutaric acid ( $\alpha\text{-KG}$ ), using free ammonia it occupies a key position in the synthesis of amino acids, and in adjusting the ratios between them, since  $\alpha\text{-KG}$  can effect the double transfer, of an amino group from one to the precursor of another. Aspartic acid and alanine can transaminate in this way but more slowly than GA, in animals at least. The enzymes concerned, the transaminases, have the B-vitamin pyridoxamine (p. 311) in their prosthetic group. Wilson (1952) has suggested that  $\alpha\text{-KG}$  might be the initial acceptor by which ammonia is fixed in plant biosynthesis and GA in fact is among the first amino acids to be formed in photosynthesis (Calvin, 1951).

Another distinct property, significant in growth, is the ready interconversion between GA and other amino acids, as entities. Histidine, arginine, ornithine and proline (Street, 1949; Kit, 1960) are readily converted to GA and some of the reactions are believed to be reversible under appropriate *in vivo* conditions. GA appears to be the main source of the alanine and glycine of silk (Sarlet *et al.*, 1952) and there are records of other, similar instances. It is possibly involved in the synthesis of nicotinic acid in some organisms (Bovarnik, 1943), and of other alkaloid bases, significant also in view of its role in the synthesis of the neurotropic amines. As a ready source of  $\alpha\text{-KG}$  it is also potential fuel for the

Krebs cycle, as already noted. It does appear to promote a number of biosynthesis reactions, including phosphorylations, purely as a source of energy.  $\alpha$ -KG can also be regarded as raw material for the formation of other members of the Krebs cycle, and members of some collateral pathways.

It may be significant also that the synthesis of GN in some micro-organisms requires a starter amount of GN itself: it is an autocatalytic reaction, once started. Also of interest is the fact that GA is the only major agent other than glucose (Mayer *et al.*, 1951) known to control the appetite centre in the hypothalamus (p. 279) and so the whole tempo of growth; this is interesting also because both are used as fuel by nerve-tissue (p. 291). The appetizing taste of GA has been noted (p. 283): since it is so important for growth, the evolution of a favourable palate for it is understandable.

GA is certainly outstanding among the a.a.s but if it were possible to consider the others in detail it would become clear that no other group of metabolites, except the B-vitamins has so many uniquely significant members.

#### 17.3.4. Other Nitrogen Compounds

With the exception of inositol all the B-vitamins are nitrogenous. They will be considered later, and likewise the purines and pyrimidines, as components of the nucleic acids. There are so many other nitrogen compounds in living animals (Baldwin, 1953) that they ought to be considered briefly if space permitted; in many cases, however, little is known of their actions on growth. In general, simple nitrogen compounds other than the amino acids appear to retard growth, some possibly through competition with the latter (Almquist, 1951). Trimethylamine is said to retard the growth of rats (Wastl, 1942), possibly by competition with choline and other quaternary ammonium compounds. However, putrescine, a dipeptide of the amino acid ornithine, promotes the growth of the micro organism *Haemophilus* (Herbst and Snell, 1948, 1949), perhaps as a source of ornithine. *Haemophilus* also demands its porphyrins ready-made; this normally presents no serious problem because it is a blood parasite. Other animals synthesize their own porphyrins (p. 243), so illustrating once more (p. 244) the essentially opportunist nature of the demands for vitamins. Urea inhibits the growth of ciliates (Kidder and Dewey, 1951) but improves that of ruminants because the rumen flora can use it as a source of protein nitrogen (Smith *et al.*, 1943). Almost all nitrogen compounds promote the growth of autotrophes, usually indirectly through the action of the micro-organisms of decay, which use them as a source of energy and degrade them to simple salts.

#### 17.3.5. Carbohydrates and Related Substances

As the source of skeletal material and of energy it would be expected that restricting the carbohydrate intake would retard growth (Lutwak-Mann, 1952), and that any increase, within the physiological range, would accelerate it. The energy supply is the more critical. Glycogen accumulates in the cells of young

regeneration buds (Needham, 1952), in hair buds (Bullough and Lawrence, 1958), and in other growing structures, and is rapidly depleted where the rate of proliferation is high. There is commonly a correlation between blood-sugar level and cell proliferation (Bullough, 1952). With insufficient carbohydrate, valuable amino acids tend to be squandered as fuel (p. 286). This may explain why increasing the sugar intake by only 0·1 per cent has been found to accelerate growth as much as a 1 to 4 per cent increase in peptone consumption (Rahn, 1934). On the other hand, extra carbohydrate is valueless if there are insufficient amino acids for building purposes, and its effect is greater on a high than on a low protein intake (Munro and Naismith, 1951, 1953).

Specific aspects of synthesis shown to be accelerated by carbohydrate include amino acid turnover in protein (Gale, 1953), and the synthesis of nucleic acids and phospholipids (Lutwak-Mann, 1952). Protein and carbohydrate together are necessary for the optimal effect on nucleic acid synthesis (Munro, 1951). Not surprisingly glucose is the most effective carbohydrate and Spratt (1948) concluded that it is the only form of exogenous energy which is absolutely essential for morphogenesis. Most carbohydrates are catabolized via glucose or at least via the glycolytic sequence, but there is some indication of alternative pathways, since in some micro-organisms trehalose and sucrose appear to promote cell division better than glucose (van Niel, 1949). L-fucose is present in human milk and seems to be a growth factor for babies. It is probably the source of the fucose of the ABO blood group antigens and of other materials. Pentoses are not used as fuel by mammals perhaps because this might adversely affect nucleic acid synthesis (p. 259). Glycerol feeds into the glycolytic system and is a good fuel; as much as two-thirds of the starch of a typical animal diet can be replaced by glycerol without deleterious effect on growth (Hanke, 1953), though it is toxic in excess. Opinion is divided on the value of lactose (Briggs and Spivey, 1954; Boutwell *et al.*, 1944); it has an inhibitory effect, particularly on the ovary of female mammals, and might have biological value in discouraging a further conception during lactation. Lactose is found to promote the growth of acidophilous organisms in the intestine, improving the absorption of Ca and P, and possibly having other beneficial effects for the nursing mammal (Brody, 1945, p. 802).

Vitamin C, or L-ascorbic acid—



is an unsaturated acid lactone derivative of hexose and in the rat is synthesized from glucose with the help of the gut flora. It is demanded as a vitamin by man, the guinea pig and other animals, including trypanosomes, but not by the rat and some other animals tested; these synthesize it in adequate quantities. It is a growth factor for young mammals (Robertson, 1923; Reid, 1950) and is

essential for embryonic development (Brachet, 1950, p. 338), and for reproductive growth processes. It is necessary for collagen formation (Kleiner and Orton, 1958, p. 302) and for that of other intercellular materials (Table 18.2, p. 304), which are particularly affected in scurvy, the classical deficiency disease of the vitamin. As already noted (p. 179), this "antiscorbutic" vitamin is essential also for the growth of bone, as a specialized connective tissue, both for fibrogenesis and for the mineralization. It may also be a direct product (Kit, 1960) of the glucuronic acid of the ground substance of connective tissues. Glucuronic acid itself proves to be a growth promotor (Reid, 1950), as would be expected in view of the amount of this set of tissues in the body. Vitamin C is very abundant in many plant tissues (Mapson, 1953), the citrus fruits being the classical dietary source. It promotes nitrogen assimilation in plants but this scarcely accounts for the particularly high content in fruits: conceivably it is an adaptation to attract animal seed dispersers!

It is a reversible oxidation-reduction agent by virtue of the enol groups on carbons 2 and 3 which become ketone groups in dehydroascorbic acid. This is still antiscorbutic so that the main function is not related specifically to either enol or ketone grouping, but it may depend on the reversible redox change as an entity, which certainly plays a large part in metabolism. Moreover, antioxidants in general are growth-promoting, as will be seen; like ascorbic acid they are reversible redox agents over a range of fairly low oxygen potential. The range of ascorbic acid overlaps that of the glutathione system—



sufficiently for both forms of the latter to act as cofactors for the ascorbic cycle (Mapson and Goddard, 1951), and so the two systems are probably coupled *in vivo*. Ascorbic acid protects vitamins A and E against oxidation (Moore, 1945; Daft, 1951) and counteracts the oxidizing effects of ionizing radiations (p. 421) and of other agents. It promotes the final stages, which are reduction reactions, in the synthesis of the B-vitamin, folic acid (p. 314). It is probably a coenzyme for a number of enzymes (Kleiner and Orton, 1958), including the cathepsins; it would be expected (p. 194) to favour the synthesis direction of action of these enzymes, just as it promotes certain industrial polymerizations (p. 195). An increased requirement for the vitamin when protein consumption is increased (Samuels, 1948) may be related to this co-operation with cathepsins.

Ascorbic acid accelerates the incorporation of acetate units into cholesterol (p. 239), perhaps by catalysing the reduction of the carbonyl groups, and this may be the main reason for its high concentration in the adrenal cortex; however, there is also evidence (p. 179) of competition between the vitamin and the formed cortical hormones, which probably act as oxidation-reduction agents themselves (Villee, 1962). Its action on synthesis in the connective tissues may be, in part at least, connected with acetylation processes. It can partly compensate for a deficiency of pantothenic acid (p. 312), which is the main component of

co-transacetylase. Administration of the adrenal corticoid, cortisone, alleviates scorbutic symptoms in the joints, presumably by reducing the demand on the vitamin for the synthesis of the indigenous hormones. On the other hand deoxycorticosterone, a member of the other main group of adrenal corticoids (p. 370), depresses the action of ascorbic acid on the growth of skeletal tissues possibly by redox action (cf. p. 361). The stresses of wounding, cold, and other deleterious agents demand an increased output of hormones from the cortex and an increased vitamin C intake. This is in addition to the increased requirement to meet repair processes in the connective tissues.

Some experiments have shown no positive effect of ascorbic acid on growth and others a definite inhibitory action (Hemingway, 1960). Shapiro (1948) found that it inhibited mitosis, perhaps an indication that this requires a higher oxidation potential (p. 260). High concentrations certainly are inhibitory (Das Gupta and Guha, 1941) but this is true of most metabolites, because the normal state demands such a precise balance. Some non-physiological unsaturated lactones are markedly inhibitory to growth (Medawar, 1940; Robinson and Heaton, 1948), but this might be through competition with ascorbic acid. A number of unsaturated aldehydes also inhibit growth.

Inositol, or bios I, hexahydroxy-cyclohexane ( $\text{CHOH}_6$ ), is an isomer of the hexoses, and itself has a number of natural isomeric forms. The active form of this animal vitamin is myoinositol or mesoinositol. It is a growth promotor in fungi (Wooley, 1944), micro-organisms, birds and mammals but not in those insects tested. It improves milk yield in mammals (Climenko and McChesney, 1942). In rodents a deficiency causes spectacled eye, which is a local alopecia, or failure of normal hair growth (Wooley, 1941; Pavcek and Baum, 1941). It is essential as a growth factor for virtually all types of cell *in vitro* (Eagle, 1960). It stimulates mitosis and alleviates the inhibitory effect of colchicine (Table 21.2), and of its own analogue, gammexane. It inhibits the growth of tumours in the mouse, a paradox which is consistent with the behaviour of some other vitamins and may be relevant to the reciprocal discovery, that carcinogens inhibit normal growth.

The essential mode of action of inositol in growth is obscured by a wealth of clues. It may be a direct precursor of ascorbic acid (Bernhauer *et al.*, 1936) though this is certainly not the main source of the latter (Kit, 1960); again there is no good evidence that it is an intermediary in the synthesis of aromatic substances from carbohydrate, via shikimic acid (Lerner, 1953). It is itself a fabric material, as the myoinositol of muscle, as the phosphatides, diphosphoinositide and lipositol, as phytin in plants and as other forms. Phytin, its hexaphosphate, acts as a phosphagen in plants, so that conceivably myoinositol has some such function in muscle. The stores of inositol in clasmobranchs in the form of scyllitol (p. 235), are thought to be merely as a fuel, though in view of the large mass of very active muscle in clasmobranchs a phosphagen function might be suspected. Somewhat surprisingly the dietary requirement for the vitamin is greater if it is in the form of phytin, but this is probably merely

because it forms insoluble complexes in the gut (p. 274). The inositol phosphatides are potential growth agents by virtue of their lipotropic, or fat-mobilizing, action (McHenry and Patterson, 1944). They cure fatty liver produced by biotin in rats, mobilizing sterols rather than glycerides. Inositol also reverses the inhibitory action of malonate on growth and so presumably affects the working of the Krebs cycle.

Structurally, perhaps, and certainly in their water-solubility, the members of the Krebs cycle are more closely related to the carbohydrates than to the fats; the oxaloacetate and some of the acetate, which feed the cycle, are carbohydrate in origin. Members of the cycle promote growth in physiological concentrations and their metabolic antagonists, such as malonate, are powerful inhibitors of growth (Rulon, 1948). The action is mainly by supplying energy but a number of syntheses also lead out from the cycle. A large number of these non-nitrogenous, water-soluble substances have been shown to promote growth, in various specific ways.

#### 17.3.6. Lipids

Under this heading may be included all non-nitrogenous, fat-soluble materials, and also all those nitrogen-containing substances, such as the phosphatides, which are fat derivatives and remain lipid-soluble. The natural biological members (p. 237) are primarily the triglycerides, or neutral fats, some hydrocarbons with a degree of oxidation and of ring closure, for instance vitamins A, E, and K, the more fully cyclized sterols, the phospholipids including simple phosphatides such as lecithin and cephalin, and the more complex cerebrosides mainly found in the myelin sheath of nerves. The glycerides are synthesized *in situ* in amounts adequate to prevent any shortage on a fat-free diet, though the unsaturated derivatives, oleic, linoleic and linolenic acids, in particular, are demanded as vitamins by mammals (Sinclair, 1952), insects (Fraenkel and Blewett, 1947) and bacteria (Boughton and Pollock, 1952); the name vitamin F has been proposed for these. The difficulty in synthesis would seem to lie in the desaturation reaction, notwithstanding the ability (p. 239) to effect this in the isoprene-like precursor of the sterols. As already noted, many insects in fact cannot synthesize cholesterol and demand it as a vitamin, while the mammals synthesize vitamin D in adequate amount only under special conditions (p. 240); that which is synthesized in the skin is made available through the preen gland secretion of birds, or through absorbing or consuming sebum by mammals. Again vitamins A, E, and K are demanded in the diet, although structurally they are based on the same isoprene unit.

Neutral fats are required almost entirely as fuel (p. 257), and they provide the most stable and economical stores of this. The natural fatty acids, with an even number of carbon atoms (p. 238), and also the corresponding monohydric alcohols, promote the growth of *Chilomonas* whereas the rare, odd-numbered members are frankly inhibitory (Cosgrove, 1951). As in the case of glycogen (p. 294), there is a tendency for intracellular lipid to be depleted so long as

growth is rapid and to accumulate when it ceases, for instance *in vitro* (Willmer, 1958). There is very little in the cells of young embryos, even of birds which have so much in their yolk, but it tends to accumulate in the adult mammal (p. 254). The view that fats inhibit growth is based largely on this kind of evidence, though there are other reasons to think that they may do so. Growth requires an aqueous medium so that lipid adequate to reduce the effective water space, or to reverse the colloid state of critical regions of the cell from an oil-in-water to a water-in-oil system could be inhibitory. In the control of phase-reversal the calcium ion and the phosphatides may play a major role. It may be significant that fat synthesis is low in proliferating tissues (p. 88).

In contrast to the variable element of Terroine, that is the neutral fats, there is a constant element which is mainly phospholipid and is an essential part of the fabric, in particular of the cell membrane and the ergastoplasmic membranes (p. 146). Steroids almost certainly contribute to this element (Booij and De Jong, 1956). The lipid is closely associated with protein, which largely masks its typical lipid cytochemical reactions. Both components increase in close proportion to the nucleic acid of the cell (Haven and Hodge, 1941; Davidson and Leslie, 1950; Cornatzer *et al.*, 1953). The membranes play a critical part in the cell's synthetic activity. Amino acids probably enter the cell as a complex with lipid (Roberts, 1960).

Free phosphatides in general, like lipositol (p. 296), might be expected to promote growth by their hydrotropic or lipotropic action on fats, rendering them soluble and more easily transported and metabolized. The results of experiments have been rather uncertain (Wilbur and Seaman, 1947) or have indicated no effect (Haven and Hodge, 1941). Robertson (1923) concluded that lecithin was inhibitory in the early stages of development but a promoter later, and conceivably this could depend on a change in significance of its ability to move between the aqueous and lipid phases of the cell. It depresses growth *in vitro* (Willmer, 1958) but promotes growth processes in the adult reproductive system (Trager, 1953), which seems to support Robertson's interpretation, since cells *in vitro* become youthful in many respects.

As already noted (p. 297) some of the unsaturated fatty acids, with double-bonded pairs of C atoms in the molecule, have the properties of vitamins (vitamin F). In addition to the evidence given, oleic acid improves the growth of fungi and micro-organisms. Vaccenic acid, an isomer of oleic, with the double bond at the C<sub>11</sub> to C<sub>12</sub>, instead of the C<sub>9</sub> to C<sub>10</sub> position, also is growth-promoting (Boer *et al.*, 1946): it is abundant in summer butter. Potency seems to increase with the number of double bonds in the molecule, bringing the structure nearer to that of the other fat-soluble vitamins. Vitamin F resembles vitamins A and E in improving the reproductive functions of animals and in being associated with pyridoxin (p. 311) in a number of its actions (Sinclair, 1952). An excess of thyroxin increases the requirement for vitamin F as well as for A and E. The action of vitamin F on growth is increased (Dam, 1951; Cheng *et al.*, 1952) by biotin (p. 310) and by the APGH hormone (p. 365). The

unsaturated fats may be components of the cell membrane (Carter and Thompson, 1953), though during starvation they appear to be catabolized along with the other glycerides.

A growth promoter released when plants are wounded, traumatic acid, 1-decene-1,10-dicarboxylic acid, HOOC-CH-CH-[CH<sub>2</sub>]<sub>8</sub>-COOH, is an unsaturated dicarboxylic acid (English *et al.*, 1939). In ciliate Protozoa the non-biological "Tweens," which are distant analogues of the unsaturated fatty acids, also promote growth (Kidder and Dewey, 1951).

Mammals synthesize some of their vitamin A (p. 240) by fission of plant carotene, but perhaps never the whole of their requirement. This vitamin promotes the growth of mammals, birds and other animals (Robertson, 1923); young animals may even lose weight under severe deficiency, and deficient mothers may give birth to eyeless and limbless young. The vitamin controls the growth of nerves by promoting the enlargement of their nerve foramina (Mellanby, 1944); blindness and deafness result when this fails and the growing nerves are constricted. Deficiency leads to overgrowth of bone around the labyrinth (Kleiner and Orton, 1958), no doubt exacerbating the deafness. The vitamin probably assists the enlargement of the cranium by promoting resorption on the inside of the table (p. 79).

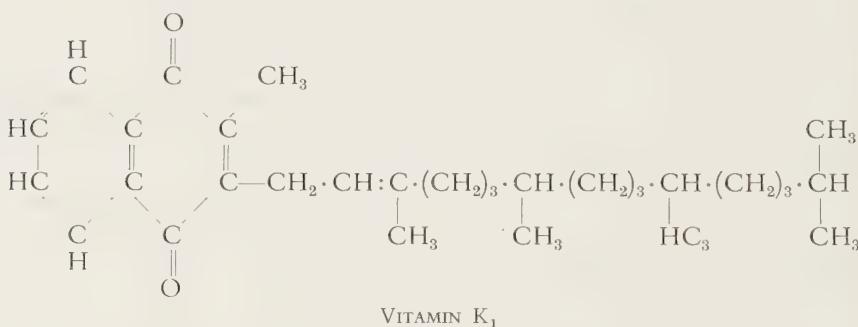
Vitamin A maintains the normal condition of the mucous membranes so that deficiency leads to xeroderma, a dry, excessively keratinizing skin (Table 18.2, p. 304); keratinization of the cornea leads to blindness. Reciprocally excess causes normally keratinizing epidermis to become mucous in type (Fell and Melanby, 1953). There may be some relation here to its action in retarding Amphibian metamorphosis (McCarrison, 1923), since keratinization of the epidermis is a metamorphic change in many Amphibia. The vitamin may also have a direct antithyroid action, since in mammals thyroxin increases the requirement for it. This is an example of the antioxidant action of vitamin A. It is also nitrogen-sparing, mollifying the effect of a dietary deficiency of protein (Moore *et al.*, 1952). Like some of the hormones (p. 369) it improves the retention not only of nitrogen but also of water, and it also improves the retention of fat (Patterson *et al.*, 1942). Like all vitamins it improves the general resistance to disease, probably by promoting the repair processes.

It is also significant as fabric material since it is the immediate precursor of the carotenoid rhodopsin, visual purple (p. 233) and related pigments. Deficiency results in night blindness, and it is interesting that this is the third, if not the fourth distinct effect of the vitamin on eye development and maintenance. A red pigment associated with growth and regeneration in the hydroid, *Tubularia*, is a carotenoid (Goodwin, 1950), and similar pigments in a number of animals appear to be associated with reproductive and other growth activities.

Vitamin E,  $\alpha$ -tocopherol (p. 240), probably often acts in synergism with A in promoting growth (Hickman *et al.*, 1944; Flückiger and Flück, 1950) and improving resistance to disease. It is best known for its beneficial effect on the growth of the reproductive structures of both sexes, including the growth of the

foetus of rodents, and the subsequent nursing behaviour of the mother. It is said, in contrast to vitamin A, to accelerate the metamorphosis of Amphibia (Wurmbach and Haardick, 1952), but by accelerating growth and not by stimulating the activity of the thyroid gland; in fact it is an antioxidant, like vitamin A, and thyroxin increases the requirement for both. In contrast to thyroxin it retards the differentiation of cells (Menschik, 1953). It is necessary for the normal maintenance of muscle, a deficiency leading to dystrophy of the tissue and an increase in respiration rate: its normal function, therefore, may be to control oxidative processes. It also confers protection against the oxidizing action of ionizing radiations, and protects the unsaturated fats from oxidation. By activating cytochrome c reductase (Dam, 1951) it assists the oxidation of the proximate substrates, keeping the oxidation potential near the lower end of its range. Vitamin E resembles A also in preventing loss of weight, improving nitrogen retention and decreasing protein breakdown (Moore, 1949; Zierler *et al.*, 1948). It forms a soluble sodium phosphate diester and in some way affects the turnover of P and of nucleic acids (Young and Dinning, 1951). It is involved with the B-vitamin, pyridoxin (p. 311), in the control of the metabolism of the unsaturated fats (p. 298).

Vitamin K, like A, E, and D, exists in a variety of related forms: K<sub>1</sub>, or 2-methyl-3-phytyl-1,4-naphthoquinone, is probably the most important. It

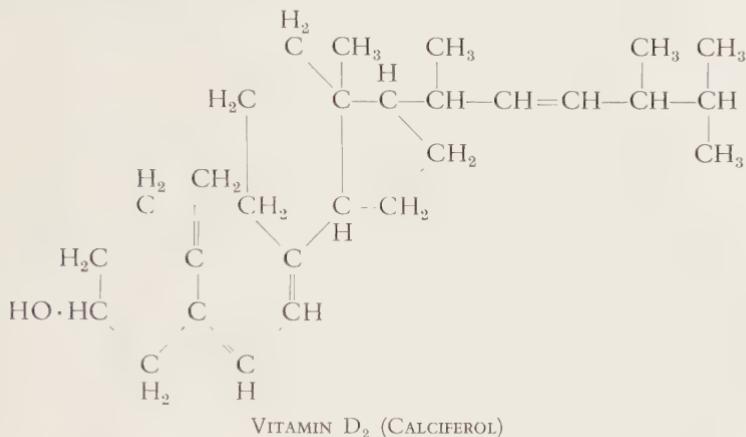


is best known for its antihaeorrhagic action on blood clotting, but it also has growth-promoting activity (Wooley and McCarter, 1940; Schweigert, 1948) and gives protection against the growth-inhibitory action of iodinin, a pigment related to pyocyanin (McIlwain, 1943). It is itself related to echinochrome, which may have oxidation-reduction functions. The vitamin has a function also in photosynthesis (Arnon, 1956). However, it has certain anti-growth effects (Glavind and Dam, 1948) and prevents the germination of some spores (Foote *et al.*, 1949), and so has antibiotic properties (Illand, 1948). Naphthoquinones tend to be antimitotic (Lehmann *et al.*, 1945).

Citrin, vitamin P, a mixture of flavone glycosides, has not been shown to have clear growth-promoting effects but like these fat-soluble vitamins it protects against the oxidizing effect of irradiation. The ubiquity of this action

seems relevant to the importance both of lipids and of oxidation phenomena in carcinogenesis (p. 107).

Vitamin D is one instance of a steroid with growth-promoting properties: others are the androgens and one group of adrenal corticoids (p. 370). The



action of vitamin D in promoting calcium deposition in the teeth and bones of vertebrates, its antirachitic action, is not entirely peculiar to it, since the sex hormone steroids also affect Ca metabolism (Gardner and Pfeiffer, 1943). The vitamin D requirement for general growth in some animals is said to be greater than that for bone growth alone, but no other growth-promoting action has been clearly recognized: from a knowledge of the androgens an effect on nitrogen retention might have been expected, and vitamin D would then have resembled the other fat-soluble vitamins, but in fact it seems to depress protein synthesis (van Lanen and Tanner, 1948). The action on bone growth includes the speeding up of Ca and P uptake from the gut, by increasing the acidity of the gastric secretion. It controls the citrate level in the body and so the amount of ionic calcium (p. 178). Provided there is adequate P the result is a deposition of Ca and P in the skeleton (Greenberg, 1943); otherwise Ca is bound as the citrate and lost to the body (Bellin and Steenbock, 1952). A deficiency of the vitamin results in rickets in growing children, and osteomalacia, softening of the bones, in adults. The parathyroid gland (p. 377) also is concerned with the Ca/P balance. Vitamin D improves the utilization of P bound in phytin (p. 296).

The insects demand cholesterol as a vitamin and it promotes their growth. Some time ago it was found to promote tumour development and the multiplication of ciliates (Robertson, 1923, p. 281), and it has now been shown essential for isolated cells of mammals *in vitro*. *In vivo*, therefore, its synthesis by mammals must be systemic or at least extracellular. Like other sterols it can also be inhibitory, probably in higher concentrations, causing fatty liver and retardation of growth in rabbits, guinea pigs and rats (Cook and McCulloch,

1939). Like the adrenal glucocorticoids (p. 369), and the female gonadal hormones it tends to deplete nitrogen in such concentrations.

Among non-biological polycyclic lipid molecules some of the carcinogens have been found to promote growth (Silberberg and Silberberg, 1944). In a sense all do so, if they induce a tumour (p. 106). The direct effect of most of them is inhibitory, however (Reese and Reese, 1945; Demerec, 1947; Haddow, 1948).

## CHAPTER 18

*The B-vitamins and Transfer Reactions*

THE B-vitamins are logically the next group of growth-controlling agents. They act at the molecular level but as agents rather than as fabric or fuel. They are required in relatively small amounts and this may be one reason why animals have come to rely on external dietary sources for their supply. Another reason is that all are usually present in the tissues of food organisms so that they are never absent from any natural, untreated diet. The universal occurrence is related to their vital role in all transfer reactions in the cell, including those of synthesis: all are essential for growth. They are in fact the essential components of the coenzymes of the transferase set of enzymes; all are therefore water-soluble and they differ from the fat-soluble vitamins in a number of respects

TABLE 18.1

**Comparison of Properties between B-vitamins and Fat-soluble Vitamins**  
 (Based mainly on Williams *et al.*, 1950).

B-vitamins	Fat-soluble vitamins
1. N and sometimes S in the molecule	Carbon, hydrogen and oxygen only
2. Vary considerably in chemical structure, though some components are common to two or more	All structurally derivable from isoprene unit
3. Mostly a unique molecule is the active vitamin	A family of related molecules are all active as the vitamin
4. Competitive analogues differ from the vitamin only in side-chain groups, i.e. activity is due entirely to particular small radicals	Competitive analogues show differences in the main carbon chain, i.e. activity is a property of the whole molecule
5. General transferase function	Special functions
6. Components of coenzymes	Not coenzymes
7. Amount in the tissues proportional to respiratory activity, i.e. to general metabolism	Storage in tissues dependent on amount in food, and independent of metabolic activity
8. Requirement for the vitamin dependent on body size (not linearly)	Requirement independent of body size; local and sporadic in action as in origin
9. Not species-specific in action	Often show high species-specificity in action
10. Usually demanded by heterotrophes	Not demanded by all heterotrophes; often synthesized in part or even adequately by them
11. Universally distributed but in limited amounts	Sporadically distributed, but often in high concentration
12. Present in microorganisms	Generally absent from microorganisms

TABLE

## Comparison of the Effects of Deficiencies of

The many similarities among these effects show that all of the B-vitamins are essential for

Vitamin						Effect of
	Skin		Hair	Nerve	Gut	
Riboflavin	Seborrhic fissures	lip	Alopecia, greying (achromotrichia)	N. trunk degeneration, curled-toe paralysis		Hypotrophy of testis
Nicotinamide	Dermatitis and keratitis			Mental and emotional disturbance	Diarrhoea and lesions; black tongue (dog)	
Thiamine				"Beri-beri," neuralgia, numbness, paralysis		
Pyridoxine	Acrodynia (rat)		Alopecia	N. degeneration, convulsions, incoordination		
Biotin			"Spectacled eye," greying			
Folic acid (PGA)			Greying		Diarrhoea, (sprue), necrosis	
B <sub>12</sub> (cobalamin)						
Choline				Paralysis	Cirrhosis of liver	
Pantothenic acid	Keratitis		Spectacled eye; greying	Spinal lesions, tachycardia, flaccid palsy		
Inositol			Alopecia, spectacled eye			
Ascorbic acid (C)	None			Lassitude		Hypotrophy of gonads
A	Excessive keratinization		Depilation	Myelin-degeneration; circling movements	Liver-lesions; diarrhoea	
E (tocopherol)				Paralysis	Liver necrosis	Testis hypotrophy; abortion (rat)
K						
P (citrin)						
D						

18.2

**the Various Vitamins on the Tissues of Mammals**

the normal maintenance-growth of all tissues (this is not true of some of the other vitamins)

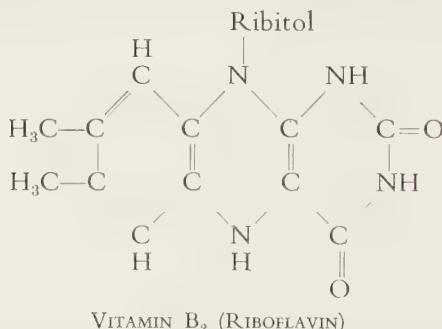
Deficiency on

Blood Cells	Lymphoid Tissues	Bone	Muscle	Other Tissues
Microcytic anaemia and leucopenia	Thymus hypotrophy	Growth retarded		Cataract of lens
Macrocytic anaemia		Anti-micromelia		Erythraemia
				Atrophy of endocrines; oedema
Microcytic, hypochromic anaemia				Oedema
		Perosis		
Macrocytic (pernicious) anaemia, and leucopenia				Hydrocephalus
Macrocytic anaemia	Increased anaphylactic reactions	Perosis		
	Thymus involution, but splenic enlargement			Slipped tendon; haemorrhages
Anaemia	Thymus involution			Adrenalatrophy
			Gut peristalsis feeble	
		Bone and cartilage dystrophy		Connective tissue (scurvy) and haemorrhages
		Resorption inhibited; limbless young		Renal calculi; pituitary changes
		Encephalomalacia; teeth affected	Dystrophy	Pituitary affected
		Teeth abnormalities		
		Rickets (epiphyses); teeth abnormal		Haemorrhages

(Table 18.1). They differ from the other water-soluble vitamins, ascorbic acid and inositol (p. 294), in containing nitrogen and sometimes sulphur in the molecule, often in heterocyclic form. Vitamin C and inositol are not co-transferases, and it is not certain that inositol is a coenzyme at all.

The B-vitamins occur together also because they interact and synergize so extensively. This is particularly evident in the maintenance aspects of growth. Deficiency of almost every B-vitamin causes much the same syndrome in adults, namely a decreased resistance to disease, dystrophy of the skin (dermatitis) and of the gut and other epithelia, and anaemia or dystrophy of the haemopoietic tissues. In some cases there is oedema of the connective tissues and perosis, or bone dystrophy, while muscular dystrophy and particularly nerve dystrophy (neuritis) also are usual (Table 18.2). In some of these features, particularly those affecting the connective tissues, ascorbic acid and inositol again resemble the B-vitamins. The interaction between the latter is shown also by the many instances where an increase in amount of one causes an increased demand for others and again by cases where a deficiency in one puts a greater strain on others. Every pathway of synthesis requires them all. In autotrophes there is the further complication that the vitamins are required at various steps in the synthesis of each other, as fabric materials. This concerns heterotrophes only indirectly, though often synthesis of vitamins by their gut flora is an important feature of their nutrition.

Vitamin B<sub>2</sub>, or riboflavin, has been shown essential for the growth of mammals, insects and protozoa, the only groups widely tested for vitamin requirements (Table 18.2). It was at first known as the growth vitamin because



it promoted growth so powerfully. Rats fed 20μg per day gained in weight ten times as much as pair-fed litter mates without the vitamin (Sure, 1941). It promotes regenerative growth (Needham, 1960a) and egg production, as well as improving the viability of the resulting chicks. The growth of bone is accelerated (Nelson *et al.*, 1947), and some individuals of *Drosophila* bearing the "antennaless" gene can be made to grow an antenna by increasing their intake of the vitamin (Gordon and Sang, 1941). It is anticarcinogenic, the carcinogens themselves being growth-inhibiting (pp. 107, 302). Deficiency of the vitamin can cause microcytic anaemia and leucopenia (deficiency of white

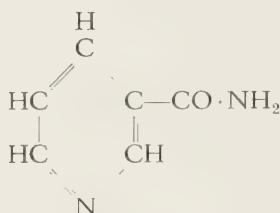
corpuscles), atrophy of testis and thymus, greying and alopecia of the hair, cataract of the lens, and degeneration of the nerve trunks, leading to "curled toe" paralysis in chicks.

Ribo-, or ribityl-, flavin (Baldwin, 1953) is a condensation product of ribitol, the C<sub>5</sub> analogue of inositol (p. 296), with 6, 7-dimethylisoalloxazine. When phosphorylated at the 5'-position of the ribitol it forms a nucleotide analogue (p. 225) known as flavin mononucleotide, FMN, or alloxazine mononucleotide. Both this and its dinucleotide with adenylic acid, flavin adenine dinucleotide, FAD, are coenzymes of a number of dehydrogenases, anaerobic and aerobic, and also of some aerobic oxidases. These directly or indirectly catalyse the oxidation of a number of the standard substrates for respiratory metabolism, in the glycolytic sequence, the Krebs cycle and other pathways. The reactions are coupled with the phosphorylation of ADP to ATP, which absorbs much of the energy released (p. 258) and canalizes its transfer to promote biosynthetic reactions. Of the substrates directly oxidized or dehydrogenated the most important are succinic acid, in the Krebs cycle, and fatty acids, but indirectly the flavoproteins control the oxidation of virtually every respiratory substrate of importance, through a sub-group known as the diaphorases. These flavoprotein enzymes act as hydrogen acceptors and catalyse the re-oxidation of the pyridino-proteins (below), which effect the initial dehydrogenation of most of the substrates in question. The flavoproteins, in turn, are reoxidized by the so-called *terminal oxidases*, the cytochrome systems, which are themselves reoxidized, in turn, by free oxygen; all therefore can continue their activity indefinitely so long as substrates are available, and the next enzyme of the series is active.

Ribityl flavin enzymes therefore promote growth mainly by dehydrogenating fuels to provide energy for the synthesis of more important molecules. Since the hydrogen is transferred from one combination to another it would be correct to call the reaction a transhydrogenation, though this term is often restricted to instances where H-reception is an actual step in a pathway of biosynthesis. The flavoproteins do catalyse reactions of this kind, though the most important are the reduction of nitrates and other oxidized nitrogen compounds to the amino stage, so that they concern autotrophes much more than heterotrophes. Reactions which do affect growth and maintenance in the latter include the synthesis of tryptophan in micro-organisms, of nicotinic acid from tryptophan in mammals, of acetylcholine from choline, of sterols (Morgan, 1951) and of visual purple from vitamin A.

The metals Mo, Cu, Fe and Mn are all cofactors for some of the flavoprotein enzymes. This variety, together with that of the apoenzyme part, gives the set of enzymes collectively a very wide range of redox potentials (Kit, 1960), from +0.80 volts for NH<sub>2</sub>OH reduction to -0.35 volts for xanthine oxidation. The four metals are shown in order of action and oxidation potential, so that Mo enzymes catalyse the initial stages of nitrate reduction and the Mn systems the final step, leading to NH<sub>3</sub> (Nicholas, 1957).

Nicotinic acid, niacin, vitamin B<sub>3</sub>, or its amide—



is necessary for the growth of mammals and Protozoa. Halogen-substituted analogues therefore inhibit growth competitively (D. E. Hughes, 1952). The vitamin itself is inhibitory in high concentration, a property by no means peculiar to this one agent. At the same time it will alleviate the growth-inhibiting action of excesses of certain of the amino acids, glycine, alanine, arginine, tyrosine, and tryptophan. In some organisms it has been definitely shown to promote protein synthesis (Kleiber and Jukes, 1942). Deficiency causes disproportionate as well as generally retarded growth; in chicks the condition is known as micromelia (Landauer, 1948; Ackerman and Taylor, 1948). In promoting the growth of the limbs especially there is a parallel to the action of ribitylflavin on the insect antenna, which is a member of the limb series.

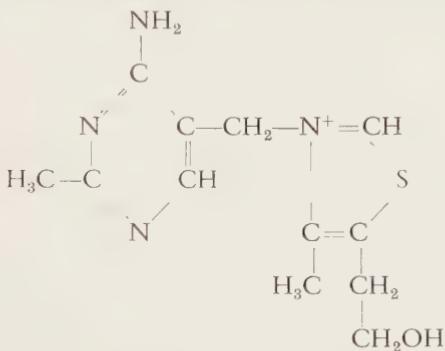
Deficiency of niacin causes a disease known as pellagra in man and it is therefore called the pellagra-preventing, or PP, factor. Pellagra is characterized by a syndrome of the three Ds: dermatitis, diarrhoea, and dementia. Symptoms common also to deficiencies of ribitylflavin and of other B-vitamins include anaemia, macrocytic in this case, and muscular weakness. Erythema, a pathological reddening of the skin, and pigmentation are common; in dogs a condition called black tongue occurs. The myelin of the nerve sheaths is abnormal.

In India niacin is synthesized by the bacterial flora of the human gut, but presumably not in adequate amounts in countries where pellagra is common; the rat synthesizes it from tryptophan (Carter and Thompson, 1953). Like ribitylflavin it forms mononucleotides, and also dinucleotides by conjugation with adenylic acid. The pyridine nucleotide is a true nucleotide, however, containing ribose and not ribitol. Like their alloxazine analogues the pyridine nucleotides form the coenzymes of a series of respiratory dehydrogenases, the vitamin itself being reversibly hydrogenated in the reaction. Again (Snell, 1953) it is difficult to attribute the growth effect to any one, or any particular group, of these dehydrogenations. All feed energy into the ADP/ATP system.

Coenzyme I (CoI, Cozymase I) is the nicotinamide adenine dinucleotide, NAD, while CoII, NADP, contains a third phosphate residue, independently attached to the ribose of the adenine nucleotide. Both catalyse a range of types of dehydrogenation. Coenzyme III is the mononucleotide, NMN, and its enzyme is probably more narrowly specific to the oxidation of organic sulphur. The enzymes have, in general, a lower oxidation potential than the alloxazine

systems and often work in series with one of the latter, in the way already indicated. Otherwise they act as fairly close analogues, and it is not surprising that the two systems are associated in the control of growth. Niacin systems show the same antagonistic relation to thyroid action. They oxidize a larger number of substrates directly and they probably promote a greater variety of biosynthetic reduction reactions than the ribitylflavin systems, particularly in autotrophes. For instance they catalyse the reduction of  $\text{CO}_2$ ,  $\text{SO}_4$ , and  $\text{NO}_3$ , of  $\text{COOH}$  to  $\text{CH}_2\text{OH}$ , of imine to amine, of  $\text{GSSG}$  to  $\text{GSH}$ , of retinene to vitamin A and of unsaturated to saturated fats. They catalyse certain reductive carboxylations and aminations in which transcarboxylases and transaminases (p. 311) also are involved. Since both groups of dehydrogenases, and particularly the pyridinoprotein group, so frequently work in reverse they could be called transhydrogenases to conform with general terminology for the B-vitamins.

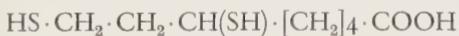
Thiamine or aneurin, vitamin  $B_1$ , is essential for the growth of mammals, insects, and Protozoa. Above the general deficiency syndrome of the B-vitamins,



VITAMIN  $B_1$  (THIAMINE, ANEURIN)

there is an outstanding neuritic effect due specifically to lack of thiamine; "beriberi" is the condition familiar in man. Both nerve cells and myelin sheath suffer, the effect beginning distally in the peripheral fibres. There is numbness (sensory) and inco-ordination, paralysis, and spasticity (motor). The alimentary tract loses its normal motility and appetite fails. The endocrine glands also suffer: this is interesting because of the functional relation between nervous and hormonal activities. Pregnancy increases the requirement for this, as for other vitamins.

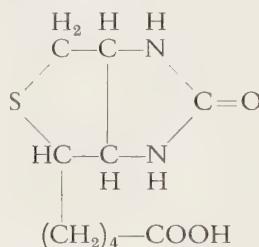
Like the foregoing B-vitamins, thiamine is possibly concerned more with energy supply than directly with synthesis reactions. In the forms of thiamine di- and tri-phosphates, it is the coenzyme of the carboxylases. Their most important reaction is the decarboxylation of pyruvate to acetate, the key reaction in the aerobic oxidation of carbohydrate. In this critical reaction the diphosphate is associated with lipoic acid (Reed, 1953), formerly thought to be a separate vitamin ("protogen"). Lipoic acid—



is active in very small amounts and in this it resembles another B-vitamin, biotin (*below*), to which it is somewhat akin structurally and which also promotes carboxylation reactions. The specificity to nerve tissue probably reflects the peculiarities of respiration in that tissue.

Thiamine promotes the conversion of carbohydrate to fat by the same key-reaction; carbohydrates therefore increase, while fats decrease, the "demand" for it. Thiamine is implicated in sterol synthesis, probably for this same reason. These are, in general, endergonic synthesis reactions, and there is some evidence that other direct synthesis reactions, including  $\text{CO}_2$ -fixation in autotrophes, are due to transcarboxylation by thiamine. It is said to retard the depletion of liver glycogen by thyroxin and therefore to favour growth, as the transhydrogenases do, by resisting excessive oxidation. A positive action on RNA synthesis has been recorded (Thompson, 1953).

Vitamin B<sub>7</sub>, biotin, bios II, or vitamin H—



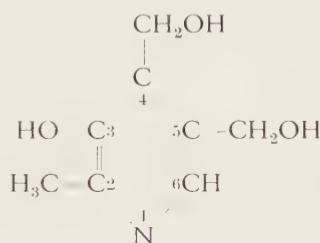
is found essential for the growth of micro-organisms, planarians (Wulzen and Bahrs, 1936), fishes, birds, and mammals, though it is synthesized by the gut flora of most mammals. Consequently a deficiency is produced only with great difficulty, by using sulphonamide drugs, or raw white of egg which contains an antagonist, avidin. Biotin promotes metamorphosis in mosquitoes and the development of chicks, and it improves milk yield. Deficiency lowers resistance to malaria and causes dermatitis, greying and alopecia of the hair of rodents, especially around the eye (spectacled eye), and the bone deformity, perosis, in chicks. It promotes cell division (Wilson and Leduc, 1949). However, it is said to enhance the carcinogenic effect of butter yellow (Du Vigneaud, 1942) which, like most carcinogens, is probably growth-inhibiting.

Again it is difficult to attribute the growth promotion to any particular reaction or action. It is one of the most potent of physiological agents and therefore almost certainly acts enzymically, and it is probably bound, in coenzyme fashion, to protein, since proteolytic agents release free vitamin. Its potency is so great,  $0.03\gamma$  ( $0.03 \times 10^{-6}\text{g}$ ) per day being adequate for the rat, that some have suggested that it is required as agent for the synthesis of one of the other B-vitamins, probably thiamine. This could explain its influence on transcarboxylations, including  $\text{CO}_2$ -fixation, in the synthesis of aspartic and glutamic acids, citric, succinic, oxaloacetic and acetoacetic acids, arginine, adenine and guanine. The manganous ion promotes the actions of both

thiamine and biotin: alternatively, therefore, biotin may be cofactor in the actual transcarboxylation reactions in the same way as lipoic acid. Biotin also catalyses certain transamination reactions and so has relations also with the next vitamin, pyridoxin. It accelerates the amination of oxaloacetic to form aspartic acid, and the incorporation of amino groups into purines, into other heterocyclic nitrogen compounds, and into ornithine to form arginine. In this last case it is linked with  $\text{CO}_2$ -incorporation, and with the formation of pseudo-peptide bonds. It is also necessary for the initial fixation of nitrogen in some autotrophes (Virtanen, 1947).

Biotin in fact resembles pyridoxin in affecting the transfer of *both*  $\text{COOH}$  (or  $\text{CO}_2$ ) and  $\text{NH}_2$ . Another similarity is in influencing either the growth functions (p. 298) of the unsaturated fatty acids or their own synthesis. Biotin, pyridoxin and thiamine are all antilipotropic, promoting the formation of fats rather than their catabolic mobilization.

Pyridoxine, vitamin  $\text{B}_6$  is, like niacin, a pyridine derivative—



but it is associated in its activity less with niacin than with thiamine and biotin. Again, it is proved to be a growth factor for all groups of animals tested, micro-organisms, Protozoa, insects, birds, and mammals. In the last, deficiency causes in varying degree the usual skin, hair and nerve dystrophy symptoms and microcytic, hypochromic anaemia. The effects are particularly evident in the paws and other extremities, a condition known as acrodynia. Deficiency in man has not been demonstrated (Carter and Thompson, 1953). It promotes the transport of amino acids and of the  $\text{K}^+$  ion across the cell membrane.

As already indicated, its particular role in the general transferase function is in transamination. Like the other co-transferases it is phosphorylated in the active state, and it becomes reversibly aminated itself, on the side-chain at position-4, in the course of the transfer. The deaminated form is pyridoxal ( $-\text{CHO}$ ) and not the common  $-\text{CH}_2\text{OH}$  form, pyridoxine. Transamination from one amino acid to the precursor of another is its most important type of reaction (p. 292), but the precursors of purines and other nitrogen bases also are aminated (Gunsalus and Tonzetich, 1952). Many of the latter can also donate  $-\text{NH}_2$ , so that their amination is reversible. Transamination to the precursors of the ketogenic amino acids occurs on the mitochondria but that to precursors of the glycogenic members occurs free in the supernatant cytoplasm

(Hird and Rowsell, 1950). This recalls the difference in location between Krebs cycle enzymes and glycolytic system enzymes (p. 262).

The pyridoxine enzymes also catalyse the initial fixation of ammonia both by autotrophes (Virtanen, 1947), and into  $\alpha$ -KG (p. 292) by mammals (Gunsalus, 1950). They also catalyse deaminations, such as that of kynurenine, during the degradative synthesis of niacin from tryptophan, and of the keto-genic amino acids to form fats (Sexton, 1949, p. 178). Food proteins therefore increase the pyridoxine requirement. The reactions of pyridoxine enzymes are commonly coupled with oxidation-reduction reactions and therefore require the co-operation of the transhydrogenases. The alloxazine systemis are known to catalyse the oxidative deamination of amino acids (Kit, 1960), and this may also be the action of niacin on excess a.a.s (p. 308). It is possibly significant that the amide is the active form of niacin. A large number of B-vitamins therefore are collectively concerned in transamination, as in carboxylation.

Pyridoxine enzymes indeed also catalyse one group of decarboxylations, that of the amino acids. Both this and the deamination of the keto-genic amino acids could favour fat synthesis, though the link so amply demonstrated between pyridoxine and fat metabolism possibly is not fully explained by this. Pyridoxine also catalyses a variety of further reactions (Snell, 1953) which are not all very evidently related to its main transfer reactions. One is the synthesis of tryptophan, from serine and indole, and another is the formation of cysteine from cystathione. The vitamin also catalyses the desulphuration and dehydroxylation of the relevant amino acids. Desulphuration of methionine could affect fat metabolism via choline synthesis (p. 239). The most important ways in which pyridoxine promotes growth would seem to be in initiating a supply of energy by deaminating and decarboxylating amino acids, in assisting the conversion of carbohydrate to fat and protein, and perhaps most of all by the variety of synthetic transaminations.

Pantothenic acid, vitamin B<sub>5</sub>—



the vitamin "found everywhere," is a growth factor for most heterotrophes tested, bacteria, fungi, protozoa, insects, birds, and mammals though possibly there is never a deficiency in man. It increases the life span of *Daphnia* (Fritsch, 1953). Deficiency causes arrest of growth, dermatitis, greying and alopecia, lesions of the spinal cord, with palsy, tachycardia and prostration, involution of the thymus and anaemia. There is an increased demand for salt but poor general appetite. The anaemia is associated with an abnormality in porphyrin synthesis, and a coloured porphyrin stains the whiskers and tears of rats. In trout there is a disease of the gills. As already noted (p. 295), vitamin C reduces the requirement for pantothenic acid and both normally have a high concentration in the adrenal cortex. There is a low concentration of cholesterol in the blood in cases of pantothenic deficiency, and the increased demand for salt no doubt is due to a deficiency of the cortical steroids concerned in salt

retention. Adrenalectomy alleviates the achromotrichia due to pantothenic deficiency, presumably by reducing the calls on the vitamin, and the bronzing of the skin in Addison's adrenal hypofunction may be related to this. Clearly the vitamin is required for steroid synthesis (p. 239).

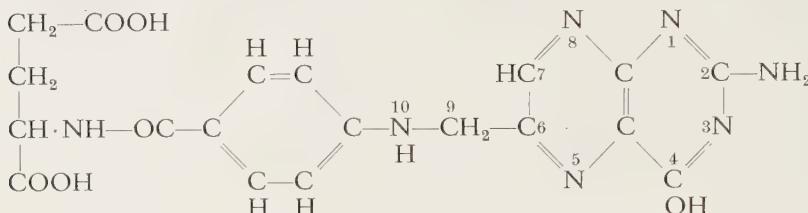
Pantothenic acid is a pseudopeptide between pantoic acid and  $\beta$ -alanine. It is part of coenzyme A (CoA or co-transacetylase). To complete the coenzyme it is further linked by a peptide bond between the COOH group of the  $\beta$ -alanine and thioethanolamine,  $\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2\text{SH}$ , the SH group being a vital part of the enzyme. The resulting compound, pantetheine, is now bonded via the  $\text{CH}_2\text{OH}$  group of the pantoic moiety through a pyrophosphate group to the ribose of adenylic acid (Novelli, 1953). It is therefore a dinucleotide analogue, if somewhat distantly. In transacetylation the SH group forms an energy-rich bond with the terminal phosphate of ATP, which is then exchanged for acetate, still with high energy available in the bond and therefore potent for various transfers and syntheses. The enzyme is required for the  $\beta$ -degradation of fatty acids and for the breakdown of pyruvate to acetate, so that again it has some control over all the significant pathways for energy release. In addition it is concerned in many important syntheses from acetate units, including those of fatty acids (from carbohydrate), phosphatides, sterols, phenolic and other ketogenic amino acids, acetylcholine, acetylglucosamine and acetyl-galactosamine (p. 235). In some organisms it promotes the synthesis of carotenoids, vitamin A and vitamin E (p. 240).

This is not the limit of its importance for growth. It will also transfer other acyl radicals, the higher homologues of the acetyl group, and such dicarboxylic acids as succinic,  $\text{HOOC} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$ ; transfer of the latter, as well as of the initial acetate, accounts for its promotion of porphyrin synthesis (p. 243). It also transfers carboxylic acid derivatives of the steroids, for instance cholic acid for the synthesis of the taurocholic acid of the bile. It transfers aromatic carboxylic acids, as in the conjugation of benzoic acid with glycine to form the excretory product hippuric acid. This involves a pseudopeptide bond and has prompted the view (p. 196) that transacetylation might play a part in normal peptide synthesis.

The synergism between transferases can be illustrated particularly well by reference to this vitamin. The isoprene-like intermediate in the synthesis of the steroids and carotenoids is a five-carbon unit probably formed in the same pathway as that of valine synthesis (Kit, 1960), from pyruvate and a  $\text{C}_2$  unit formed by decarboxylation of pyruvate. Carboxylase and transacetylase are necessary for this step, and CoA is required for the  $\text{C}_3 + \text{C}_2$  conjugation. Dehydrogenases also are active because the reaction is coupled with oxidation. Moreover, an acetyl residue can be subsequently conjugated with other molecules via either of its two carbon atoms. The one requires a transacetylase and the other a transmethylase (*below*). Acetate is often broken down to  $\text{C}_1$  units for further use. If we consider AMP to be a B-vitamin (p. 317) then the use of ATP to activate CoA is another type of vitamin synergism. A third type of

interdependence is illustrated by the need for folic acid, the vitamin next to be considered, in the synthesis of pantothenic acid itself.

Folic acid, folacin, or pteroylglutamic acid (PGA) is proved to be a growth factor for all groups of animals tested. It also promotes the process of pupation



FOLIC ACID (FOLACIN, PTEROYLGLUTAMIC ACID)

in the mosquito, and the development of eggs in the ovary. It promotes cell division, which is inhibited by analogues of the vitamin and by other anti-PGA agents (Jacobson and Webb, 1952). Among the usual deficiency symptoms, macrocytic anaemia is outstanding, and it is therefore known as the anti-anaemia factor. However, in this activity it is very closely associated with vitamin B<sub>12</sub> (below) and the latter may be the actual antianaemia principle. Folacin deficiency also causes leucopenia. The vitamin improves nitrogen retention by the body (Grubbs *et al.*, 1948), just as does glutamic acid itself (p. 291).

The pteroyl moiety of the vitamin is a pteridine conjugate of *p*-aminobenzoic acid which, in turn, is peptide-bonded to the  $\alpha$ -NH<sub>2</sub> group of the glutamic acid. *p*-Aminobenzoic acid may occur free in some organisms but in most it has much the same effect as the whole vitamin of which it is therefore the most significant component. For some bacteria (Davis, 1950) its analogue *p*-hydroxybenzoic acid has the properties of a vitamin. It is of interest, and no doubt of significance, that the pterin nucleus is a purine analogue and also is structurally part of the isoalloxazine nucleus of ribitylflavin (p. 306). The structural relationships between niacin and pyridoxine, and between lipoic acid and biotin have already been pointed out. In addition thiamine contains the pyrimidine nucleus and there are many other structural links among the B-vitamins and between them and the nucleic acid nucleotides. No doubt a detailed explanation of these resemblances would go a long way towards explaining the whole transferase aspect of biosynthesis. Soluble RNA is a set of specific amino acid transferases (p. 196), perhaps effectively the prosthetic groups of the a.a.-activating enzymes.

The active form of the vitamin is hydrogenated at the 5, 6, 7, 8-positions (tetrahydrofolic acid) and formylated at the 5-position of the pteridine nucleus or at the 10-position, that is at the NH group of the *p*-aminobenzoic moiety. The 5-formylated compound, known variously as folinic acid, leucovorin, CV-factor or CF-factor, is often a thousand times as active as folic acid itself, but in some cases the 10-compound is the most active (Kit, 1960). The active

form is coenzyme for the transfer of C<sub>1</sub> units, usually in the formyl state. Once more, therefore, the coenzyme forms an addition compound with the radical it transfers. Hydroxymethyltetrahydrofolic acid also seems to be an active form, indicating that C<sub>1</sub> units are transferred in the —CH<sub>2</sub>OH state also. The coenzyme is usually designated CoF but a polyglutamic derivative, CoC, may be the active form in some cases. It is likely that the growth-promoting action of PGA depends entirely on its C<sub>1</sub>-transfer activities, which have a very wide application in biosynthesis. The enzyme will catalyse the fixation of free formate in some organisms.

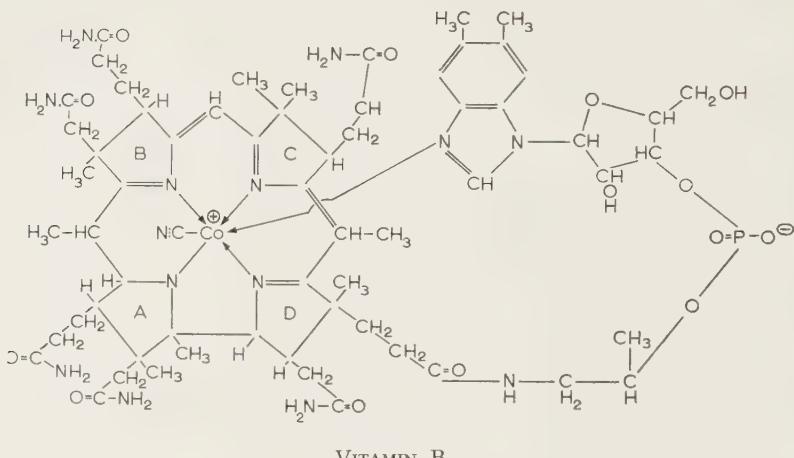
C<sub>1</sub> units at these levels of oxidation are used extensively in the synthesis of purine and pyrimidine bases, and the vitamin is found to be particularly important for the synthesis of nucleic acids; it counteracts the inhibitory effect of aureomycin on the synthesis (Rege and Sreenivasan, 1954). Through this control of nucleic acid synthesis it affects the early stages of erythropoiesis, and this is why the anaemia resulting from folic deficiency is of the megalocytic type. Porphyrin synthesis itself does not depend on C<sub>1</sub> units in these states. The promotion of CoA synthesis by this vitamin is at the stage of formylating isovalerate to form pantoate (Popp and Totter, 1952).

Vitamin B<sub>12</sub> is a growth factor for protozoa, birds and mammals, including man. It also improves the production of eggs by hens and the fertility of the eggs (Almquist, 1951). Deficiency in adults leads to anaemia, in particular, and it appears to be Castle's extrinsic factor, which combines with an intrinsic factor, necessary for its absorption from the gut. This is a glycopeptide, so that the association in the complete haematinic principle may be comparable to that between co- and apo-enzyme. Another deficiency symptom is perosis, also seen in cases of biotin and choline deficiencies.

Like hydrofolic acid, vitamin B<sub>12</sub> is a mild reducing agent and it counteracts the inhibitory effect of thyroxin on later stages of growth (Shive, 1951). Also like folic acid, it improves nitrogen retention and so increases body weight (Welch and Nichol, 1952); consequently an increase in dietary protein increases the demand for the vitamin (Almquist, 1951; Emerson and Folkes, 1951). It is not clear how far this is related to nucleic acid synthesis which the vitamin also promotes, again in the same way as folic acid. Benzimidazole riboside, an analogue of vitamin B<sub>12</sub>, inhibits RNA synthesis (Allfrey *et al.*, 1957) and the vitamin alleviates the inhibitory action of aureomycin, though it is less effective in this than PGA. The action is primarily on purine and pyrimidine syntheses, and it is clear that the co-operation between the two vitamins is particularly close. B<sub>12</sub> and PGA are both lipotropic also.

Much of the present evidence would be consistent with B<sub>12</sub> acting as cofactor with CoF in C<sub>1</sub> transfer, though its structure is very different from that of PGA and might be more in favour of a distinct function, perhaps in the synthesis of PGA itself (Welch and Nichol, 1952); if so then it would be correct to regard PGA still as the essential antianæmia factor. The structure of B<sub>12</sub> is particularly complex (Hodgkin *et al.*, 1955) and its elucidation a rather recent triumph.

It is a cobalt porphyrin, doubly linked to a benzimidazole nucleotide, through the phosphate group and through the imidazole base. The cobalt ion forms the usual chelate compounds with such groups as CN, NO, and OH, but while the haem analogy might explain its reducing properties it does not immediately throw light on those of C<sub>1</sub>-transfer. There are many methyl groups in the molecule, and this at least may be significant since there is some evidence that B<sub>12</sub> might be concerned with the transfer of C<sub>1</sub>-units in the —CH<sub>3</sub> state. The presumption is that CoF transfers units in the more oxidized state, and that the close synergism between the two vitamins is due to interconversion between the different states. B<sub>12</sub> promotes the synthesis of choline, by the repeated methyla-



tion of glycine (Stekol *et al.*, 1953), and liver transmethylase activity is low in B<sub>12</sub>-deficient rats. The reducing activity would be useful in converting C<sub>1</sub>-units to CH<sub>3</sub> and in maintaining this state; it is found that other reducing agents, vitamins E and C, and also GSH (p. 287), can deputize for B<sub>12</sub> under certain conditions (Welch and Wilson, 1949), or potentiate its action. There are cases where PGA appears to be involved in CH<sub>3</sub> transfer but this might be indirect, through the interconversion mechanism; at the same time PGA also is a reducing agent and there are some transmethylations which appear not to require B<sub>12</sub>. If the activity of the latter were restricted to special transmethylations, for instance in the synthesis of choline and methionine, it would still be a very important methylating agent, directly and indirectly. It has been suggested that B<sub>12</sub> might be concerned with the transfer of heteromethyl groups, attached to S or N atoms, and PGA with that of CH<sub>3</sub> attached to another carbon atom. Some transmethylations are anaerobic (Challenger, 1951) and these might be specific to B<sub>12</sub> as the stronger reducing agent.

In those organisms which can synthesize methionine (p. 289), vitamin B<sub>12</sub> is involved not only in the transfer of the  $\omega$ -methyl group but also in keeping the

sulphur atom of the precursor, homocysteine, reduced. Through transmethylation it affects fat metabolism, in somewhat the same way as pyridoxine (p. 311), and it is perhaps significant that the latter also controls sulphur oxidation. Both are involved in the conversion of ketogenic amino acids to fats and the large number of amide endings to the side chains in the molecule of  $B_{12}$  may imply that it has some significance in the transamination step. Fats improve the action of  $B_{12}$  (Emerson and Folkers, 1951), which is essentially lipotropic, or fat-mobilizing; this probably depends specifically as shown below, on its promoting choline synthesis, by the methylation of glycine.

Choline, a quaternary ammonium compound, itself is often regarded as a B-vitamin, since it is essential for growth in mammals, birds and insects, for lactation in mammals and for egg production in hens. It is usually required in amounts greater than those synthesized *in situ*, and it has a number of properties typical of the B-vitamins. Deficiency causes paralysis, thymus involution, haemorrhages and slipped tendon as well as other common dystrophic effects. More specific to choline are splenic enlargement and cirrhosis of the liver. It is also anticarcinogenic (Boyland, 1948).

Its ultimate action on growth processes may be twofold, first as a donor of methyl groups for various syntheses, including that of the phosphagen, creatine (p. 244), and secondly as itself a component of definitive metabolites. Lecithin and other phosphatides constitute one important group of such metabolites. Lecithin is one of the most powerful lipotropic agents in the body, by virtue of its solubility in both aqueous and lipid media; it is a substituted glyceride (p. 239) and this may explain why choline cures fatty liver due to neutral fats but not that due to cholesterol, in direct contrast to inositol (p. 297). Presumably in this same indirect way choline promotes the oxidation of straight-chain lipid molecules (Artom, 1953). Acetylcholine is a second definitive metabolite with significance for growth (p. 355). Carnitine, another quaternary nitrogen compound, also has the properties of a vitamin (Leclercq, 1954; Fraenkel, 1954). Methionine (p. 289) is probably the ultimate methyl donor for most transmethylations (Florkin, 1960). For this it is activated by ATP, with which it becomes conjugated through its sulphur atom, forming S-adenosylmethionine.

Adenylic acid, AMP, is often considered to be a B-vitamin on the same general grounds as choline. Like choline it is probably synthesized by all animals, but often inadequately during rapid growth. It has been seen that AMP is required as material not only for incorporation into the nucleic acids but also for a number of the coenzymes formed from the B-vitamins themselves, as well as for ATP, which must be subject to a heavy loss by wear. As would be expected, therefore, AMP is required in much larger amounts than the other B-vitamins, except choline; the two are possibly more in the nature of substrates for biosynthesis reactions than of coenzymes, although AMP does become incorporated into many of these. However, because its methylation is readily reversible choline has essentially transferase properties, like most of the

B-vitamin coenzymes. AMP could be regarded, like most of the B-vitamins in their usual free form, as merely the precursor of the active coenzyme, which in this case is ATP; ADP is rephosphorylated repeatedly and ATP might be regarded as co-transphosphorase, the apoenzyme being ATP-kinase. The association between the two may be temporary but there is, in fact, considerable variation among the transferases in the permanence and rigidity of the link between their co- and apo-enzymes.

The growth of rats is stimulated in direct proportion to any supplement of ATP (Garattini and Paoletti, 1954), and inhibitors of ATP formation or of its subsequent action also inhibit growth. It is already clear that there is scarcely a reaction or other component of the growth process which does not depend on ATP, usually quite directly, for its supply of energy, and only a summary is necessary here. Its first main function is that of phosphate donor, as already suggested: the phosphate may be transferred to either energy-rich or energy-poor combination, or even freed as inorganic phosphate (Chapter 13). In the first case the energy still available in the bond is usually more important than the phosphate radical itself. Carbohydrates and many of their derivatives are usually phosphorylated in their metabolically active state. The B-vitamins are virtually all phosphorylated, and other examples include vitamin D (Zetterstrom, 1951), vitamin E and other alcohols, including inositol. The second main function is in activation, for instance of amino acids, nucleotides and acyl groups (p. 196). Here the AMP moiety remains bonded to the activated molecule and a pyrophosphate residue is set free. It is perhaps correct to recognize a third function in which the high energy of the pyrophosphate links is made available for synthesis or other work, without the formation of conjugates with either P or AMP, though in most cases such compounds probably are formed, directly or indirectly. This is probably true even for apparently physical processes such as the intake of amino acids into the cell.

The uniqueness of ATP has been discussed by Lipmann (1951, p. 521) in particular. It lies mainly in a combination of thermodynamic lability, in virtue of the energy available from P bonds, with kinetic stability—the very low rate of spontaneous rupture of the bond. Many other compounds in the body have the  $\sim P$  bond but none has such great kinetic stability. A good contrast is provided by acetic anhydride, a compound similarly releasing considerable energy when hydrolysed, but doing so spontaneously and very rapidly.

The rephosphorylation of ADP or of AMP also is vital for growth. Agents which uncouple respiratory oxidation processes from this rephosphorylation, dinitrophenol (DNP), thyroxin analogues and azides in particular, are found to inhibit growth and constituent processes. Examples are the growth, moulting and pupation of insects (Thornton, 1949), amino acid incorporation and fat synthesis. Agents which inhibit the subsequent turn of the cycle, the breakdown of ATP, equally inhibit growth. Beryllium salts act in some such way (p. 179; Hoagland, 1952) and powerfully inhibit growth (Needham, 1941; Thornton, 1949; Chevremont and Firke, 1951).

There may be undiscovered B-vitamins (Folkers, 1949) and effects of unidentified factors continue to be recorded, though naturally the most important were recognized early, because of their striking deficiency symptoms (Table 18.2, p. 304), and there are probably few undiscovered ones of major interest (Williams *et al.*, 1950). Collectively the known members control the transfer of most of the small radicals which are significant in metabolism. The number of these, and of the B-vitamins, is not great but biological molecules are reiterate combinations of these few. For instance the fatty acids are polymers of the acetate unit alone. Among common radicals the OH group is outstanding in having no known transferase, though it is transferred in connexion with phosphorylations, hydrations and other reactions. Possibly it is automatically transferred with the other moiety of the molecule from which it comes, though a specific enzyme would be expected in view of the great specificity of other transfers, for instance that of C<sub>1</sub> units; different enzymes are required to transfer the COOH (or CO<sub>2</sub>), HC:O, CH<sub>2</sub>OH and CH<sub>3</sub> states of the unit. The SH group is possibly another example. Both pyridoxine and vitamin B<sub>12</sub> appear to control transfer reactions of the group, and in some cases pyridoxine seems to be a genuine desulphydrase, but this could be due to synergism with a more specific enzyme. Pyridoxine sometimes acts as a dehydroxylase also (p. 312).

The scope of action of the transferases is greatly increased by their ability to transfer large molecules attached to their essential radical: CoA is a good example (p. 313). Similarly pyridoxine catalyses the fission of cystathione, kynurenine and tryptophan into moieties larger than the amino and carboxyl radicals themselves. However, there are enzymes, such as thiaminase, which seem quite specific to the two large moieties in their entirety.

In autotrophes the transferases collectively must be autosynthetic, since they also contain only these same common radicals in their molecules. The collective mechanism must be rather complex (Kit, 1960). Moreover no one member can safely be autosynthetic in isolation (Hinshelwood, 1956), and there is probably collective control of orderly and proportionate syntheses. This component of the interaction between these vitamins further complicates an already complex situation, and the heterotrophes should be valuable, simple subjects for study, because, in general, they do not synthesize their own vitamins. Unfortunately they do synthesize some of their AMP and choline, and there is no guarantee that they do not synthesize the others in limited amounts. The elimination of their gut flora is now a simpler problem: this flora otherwise synthesizes B-vitamins, which become available to the host.

### **18.1. Control of Biosynthesis by Substrates and Products of Reactions**

Typically the action of the transferases is to speed reactions and not to exercise inhibitory control. In principle, restrictive control could be imposed quite effectively at higher levels in the Metazoa, and a number of the agents acting at higher levels are of that kind. However, there are also some suppressive

controls at the reaction level; in general each is the end product of a metabolic pathway and acts by negative feedback (Pardee, 1956; Magasanik, 1958; Krebs, 1959; 1962; Davis, 1962). Davis calls the phenomenon *end product inhibition* or *retroinhibition*. Usually this action is not directly on the final step which gives the controlling product but back on the first reaction of the whole pathway, so that the entire pathway is blocked and there is no accumulation of intermediate compounds. The control is exerted on the activity of the enzyme, and not on its synthesis as in the case of the repressor systems (p. 211). It may be visualized as making the rate of a pathway of biosynthesis steady throughout growth and as arresting it completely at the end, when an accumulation of end products may become permanent. It is probably capable also of regulating growth rate in the face of fluctuations in the supply of raw materials.

Cytidyllic acid controls the pathway of synthesis of the pyrimidine nucleotides in this way (p. 224), and similar controls operate in the purine and other pathways. Virtually all the amino acids control their further synthesis in this way (Roberts, 1959; Umbarger, 1962) in those organisms which normally synthesize them. The bile acids likewise control their further synthesis from cholesterol (Krebs, 1962), and the latter controls its own synthesis from the C<sub>5</sub> precursor (p. 239). In this case some of the intermediary compounds in the pathway, for instance mevalonic acid, squalene and lanosterol also control in the same way, and Jacob and Monod (1962) therefore prefer the term *allosteric* to end product inhibition. The term emphasizes the very important fact that the suppressor is not structurally related to the substrate of the enzyme which is actually inhibited. They regard the inhibition therefore as an example of biological opportunism, an autonomic controlling device, perfected through natural selection, and not a chemically automatic device like the mass-action effect. It is an example of the "streamlining" or superficial simplicity achieved through natural selection (Needham, 1937).

The hexose phosphates may control further hexokinase activity in this way (Krebs, 1962), and in fact it seems likely that every pathway, anabolic and catabolic, has this type of control. The individual pathways are remarkably free from interference by others even when they have intermediates in common. Thus lysine suppresses its own further synthesis without affecting those of methionine and threonine (Davis, 1962). There are sometimes two enzymes for one and the same reaction, in the same cell, when this reaction occurs in two different pathways; only one of the two isodynamic enzymes is affected by suppression of one of the two pathways.

As a result of this kind of control the amount of idle products and intermediates which accumulates is infinitesimally small (Jacob and Monod, I.c.) and this is one reason why mass-action effects are not so important in living organisms as might have been expected. An interesting exception is in the action of alkaline phosphatase, which has a high affinity for inorganic phosphate (P<sub>i</sub>), the product of its action, and so forms a complex which does not react with the substrate. There are other examples, of course: glutamic acid promotes its

own oxidation by glutamic dehydrogenase, while in the presence of  $\text{NH}_3$  and  $\alpha$ -ketoglutarate the reaction proceeds in the reverse direction. Again, oxygen is not used for the phosphorylation of ADP to ATP unless both ADP and  $\text{P}_i$  are in adequate amount (Krebs, 1962); as a corollary, if either of the two is in limiting concentration it controls the whole process. In low concentration a.a.s promote protein synthesis, while in higher concentration they stimulate RNA synthesis also (Magasanik, 1959); the action on protein synthesis may be mass action but that on RNA synthesis (Stent and Brenner, 1961) is probably due to enzyme induction (p. 207).

There are thus a number of types of control at this level and at present it is not always possible to say which type is operating in any particular case. For instance, so long as glucose is being used for glycolysis any available free fructose-6-P is not drawn into the process, although it is an actual intermediate in the glucose pathway (Roberts, 1959). Nevertheless it will donate P for use in glycolysis and inhibit the use of  $\text{P}_i$  for the purpose. Again, if there is an abundant supply of glucose, energy is produced by glycolysis and the Krebs cycle can be used in the direction of synthesis (p. 237), but if the main fuel available is acetate or succinate it must be oxidized in the Krebs cycle, and this cannot then be used for synthesis, while the glycolytic system can (Davis, 1962). These are ingenious but still rather obscure mechanisms.

The induction of enzymes was regarded (p. 207) primarily as an example of protein synthesis, but it is also a very important type of control in the present context, since the substrate is the main inducing agent. As already suggested (p. 214) the amount of some of the enzymes controlling synthesis, and in autotrophes probably also the amount of the B-vitamin co-transferases themselves, may be controlled in this way; their activity is controlled in consequence.

In balanced opposition to enzyme induction by the substrate there is the phenomenon (p. 211) of enzyme *repression* by intermediary products (Davis, 1962). It can be regarded as an alternative to end-product inhibition, but differs in being an inhibition of the actual synthesis of the enzyme and not directly of its activity. Also, the power of repression is not so narrowly restricted to the final product of a pathway. Repression is less sensitive to slight changes in the concentration of the end product than suppression and causes a more gradual as well as indirect arrest of enzyme activity. It should prove to be a more long-term device. The best known example is probably the "glucose effect" (Magasanik, 1962), by which the accumulation of intermediates in the glycolytic sequence causes repression of the further synthesis of enzymes for the pathway, which otherwise would exacerbate the condition.

The mechanism of enzymic repression/induction permits a very labile shift between pathways (Kornberg, 1962), according to the raw materials available. Whatever the nature of this substrate the organism is able to operate at least one of the available exergonic pathways for respiration and one for anabolism. These mechanisms are probably more important in microorganisms than in the higher Metazoa, which have hormonal and other controls at higher levels.

However, the control of hormone production has something in common with the present type of mechanism.

Rather logically it has been suggested that the quantitative control of regenerative growth depends on this kind of feedback mechanism and that cancer is due to its failure (Davis, 1962). In metazoa it seems probable that systemic controls play the major part but, on the other hand, regeneration and the initial cancer are local growth processes: some of the control therefore is likely to be of the present type. In fact there are devices for isolating the early regenerate (Needham, 1952; Singer *et al.*, 1955).

It is evident that this field is one destined to contribute a great deal to our understanding of the control of growth, and of metabolism in general. The leading questions are: how do suppressor products inhibit enzyme activity, how do repressor products act on the regulator genes (p. 212) to produce the definitive repressor agents and how do these actually repress the genes which promote enzyme synthesis? These metabolic control mechanisms are as purposive in their effects as behaviour patterns at higher levels of organization, and Davis (1962) has applied Pittendrigh's term *teleonomic*—intended to forestall any charge of teleology! The extent of control at this level may not be so great in metazoa as in micro-organisms, but it is enough to give the impression that every component from the meanest substrate upwards, is an active agent in the control of growth.

## CHAPTER 19

### *Nucleic Acids in the Control of Growth*

IT has now been established beyond any reasonable doubt that nucleic acids play a dominant part in their own synthesis and in the synthesis of protein, and therefore in the general growth of the organism. Much of the evidence is given in various contexts throughout the book and it is necessary here only to summarize the position. It has also been established that both forms, DNA and RNA (p. 220), promote protein synthesis, RNA being the more important as measured by the amount of protein produced. DNA alone is active in some viruses, which contain no RNA (Burton, 1955), and may be the more important in some bacteria (Gale, 1956); in all cells it is the more important within the nucleus (Allfrey *et al.*, 1957). RNA is the sole agent in those viruses which lack DNA, and in the cytoplasm of cells in general; it probably contributes to protein synthesis even in the nucleus (Brachet, 1954). Because of the larger absolute amount of synthesis in the cytoplasm this has received most study.

The first important discovery (Wilson, 1928, p. 658) was that the growth of the protozoan cell ceases soon after enucleation and this has been amply confirmed in recent years (Brachet, 1954). Meanwhile RNA disappears from the cytoplasm, presumably destroyed through wear, since it would continue to give its typical reactions if it had merely become incorporated into nucleoprotein (p. 231). The concentration of RNA is consistently high in cells growing or proliferating rapidly, microorganisms (Gale, 1953), embryonic and neoplastic cells, cells of root tips (Chayen and Norris, 1953) and cells of regenerating structures. It is high in the thymus of young mammals, but not in the spleen until this begins to produce lymphocytes. It is high also in cells producing protein, for instance pancreas acinar cells, silk gland cells and oocytes (p. 124). There is much RNA in peptic cells but little in the oxyntic cells, which produce only HCl. The concentration is low in resting yeast cells, and in mammalian erythrocytes once haemoglobin synthesis has ceased.

The high NA content has been related specifically to protein synthesis in protozoa (Jeener, 1952), in cells *in vitro* (Hull and Kirk, 1949), in feather buds (Grenson, 1952) and in cells where enzymes are being produced (Spiegelman, 1950). Stimulation of a nerve leads to the synthesis of both NA and protein in the neuron, and both begin to appear at the stage of gastrulation in amphibian embryos. NA synthesis is active in autotrophes when fixing nitrogen into amino acids (Abrams *et al.*, 1949): the fixation leads on to protein synthesis.

There is good evidence that the correlation is a causal one. Destruction of RNA by the specific enzyme ribonuclease inhibits the growth of roots, the

multiplication of viruses, the incorporation of amino acids (Brachet, 1957) and the synthesis of protein (Fowden, 1957). In those special cases where DNA is the only active form of NA the enzyme DNAase, but not RNAase, is found to inhibit growth and protein synthesis (Chantrenne, 1961). However, even where RNA is the active form DNAase may still inhibit growth, and added DNA may speed a.a. incorporation (Wood and Berg, 1962) because DNA is required for the initial synthesis of RNA (p. 227) and because DNA replication is essential for cell proliferation and sustained growth. It is probably for the first reason that DNA is found essential, in addition to RNA, for enzyme induction (p. 217). DNAase does not inhibit a.a. incorporation if there is already adequate RNA.

Experimentally administered RNA, and its constituent nucleotides, nucleosides and bases, in suitable concentrations have all been shown to promote protein synthesis and growth, in various organisms and tissues. As an example of this, additional RNA promotes the incorporation of amino acids into the proteins of bacteria (Gale, 1956) and the growth of heart fibroblasts *in vitro* (Tennant *et al.*, 1942). Nucleoprotein preparations also have been shown to promote growth *in vitro* (Fischer, 1946; Brachet, 1950; Willmer, 1958), and regenerative growth in liver and skin (A. F. W. Hughes, 1952b). Nucleotides and nucleosides stimulate additional growth in yeast (Loof bourow, 1948) and in *Entamoeba histolytica* (Nakamura, 1957), and the proliferation of blood cells *in vivo* (Doan, 1932). The purine and pyrimidine bases, and their precursors, such as hypoxanthine and orotic acid, have been shown by a number of experiments to improve growth (Hinshelwood, 1944; Makino *et al.*, 1953; Loftfield, 1957), while competitive analogues of the bases inhibit it (Brachet, 1957, p. 237). In higher concentration nucleic acids (Needham, 1952) and their components inhibit growth but we have learned to expect this of all significant materials. The bases and their derivatives may sometimes have carcinogenic and other abnormal effects (Parsons *et al.*, 1947). The purine base, *kinetin* (6-furfurylaminopurine), has a special function in growth (Kennell, 1960), but probably as a free agent, not combined in polynucleotide form.

In a number of instances it has been shown that protein synthesis continues when that of NA has been arrested by irradiation or by uranyl chloride treatment, or when there is no turnover of NA (Danielli, 1953, p. 118), or a decreasing rate of turnover (Hokin and Hokin, 1954). This has been taken as evidence that nucleic acids are not indispensable for protein synthesis, but other work has shown that the presence of NA in active form is the sufficient condition, without the necessity for further synthesis, except to replace wear. In viruses (p. 153), in microorganisms (Jeener, 1952) and in liver regeneration (Needham, 1960a), NA synthesis in fact normally precedes that of protein although the two phases may overlap considerably. Jeener (l.c.) and others have shown quantitatively that the rate of protein synthesis is related to the prevailing concentration of NA and not to its rate of synthesis. In cells growing by hypertrophy (p. 21) protein synthesis regularly outstrips that of NA towards the end of the growth cycle; in brain and liver it becomes four times as fast (Brachet, 1952).

In some cases protein synthesis appeared to continue with RNA completely inactivated (Chantrenne, 1952*b*), as measured by phosphorus incorporation. However, it now appears (p. 189) that materials may show no metabolic turnover while they are in their most active functional condition (Hogness *et al.*, 1955; Pardee, 1954; Ebert, 1954*a*), and also subjected to minimal wear.

Accepting the view that the rate of protein synthesis is related to the amount of functional NA present, the probability is that the latter is acting as a catalyst, rather than as a component of the molecule being synthesized; one implication would then be that if nucleoprotein is an intermediary in protein synthesis (p. 231) then it is a very transitory one. Simultaneous synthesis of NA and protein is not to be expected, though it is recorded in some cases (Novelli and Demoss, 1957) and certainly there must usually be some overlap, protein synthesis beginning before that of NA is complete. Synthesis of NA should, and usually does, begin first and there is no very good evidence that it has any other function than as catalyst for protein synthesis, and perhaps also for proteolysis: Binkley (1954) found dipeptidase to be almost 100 per cent RNA. Further, DNA is significant almost entirely as the genetic material for continuing the same catalytic function in subsequent generations of cells and individuals; it can do so in foreign individuals through the interesting phenomena of transformation and transduction (McElroy and Glass, 1957). The purine analogue, 8-azaguanine has been found to inhibit protein synthesis more than RNA synthesis (Otaka, 1960); this apparent paradox is to be expected if the NA acts catalytically, since a small deficiency of the catalyst can cause a much more dramatic deficiency in the product of the process.

It seems that there are two distinct catalytic roles of RNA, that of the free carrier NA in the supernatant cytoplasm and that of the ribosome-bound NA which probably acts as a heterotemplate controlling the condensation of a.a.s in a specific peptide sequence. In *Escherichia coli* there is an indication that different parts of the template RNA molecules are required for the incorporation of the various a.a.s (Gale, 1956). In this template role, if not also in that of a.a. carrier, the NA is acting as a controlling agent of a distinctly higher order (Prescott, 1961) than that of the transferases of the previous chapter. Here larger units, the a.a. molecules, are being transferred, and the template has a very specific micromorphological organization which it translates into the protein synthesized. It is significant that nucleic acids are not required for the synthesis of glycogen, which is an almost monotonous repetition of glucose units (Mercer, 1958), and the RNA-bearing ribosomes are very sparse in cells synthesizing glycogen.

There is some reason to think that only a small part of the particulate RNA may function as active templates. It is called *messenger RNA* (Brenner, 1962; Gros *et al.*, 1962), because it is thought to carry the transliterated code for the synthesis of a particular protein from the relevant nuclear gene to the ribosomal site of actual synthesis. Except that uracil replaces thymine, and ribose the deoxy-sugar, it is an exact complement (p. 227) of the genetic DNA (Spiegelman,

1962; Hurwitz *et al.*, 1962), whereas the other, cytoplasmic RNA differs from this and is very variable in its base ratios. Messenger RNA is very unstable and must be continuously synthesized so long as protein is to be synthesized; the corresponding nuclear gene therefore must be continuously active. This conflicts with the conclusion, above, that protein synthesis does not always demand continuous RNA synthesis, and some authorities believe that messenger RNA is converted into a more stable form which is still an active template (p. 334).

It is a point of great interest that the soluble RNA, by contrast with all other NA fractions in the cell, contains a number of the more unusual pyrimidine and purine bases (Sinsheimer, 1962) and occasionally an unusual pentose sugar. The significance of this is not yet clear: there is a different soluble RNA molecule for each a.a. transferred, and it is possible that they play some part in arranging the a.a.s on their template in a specific order. However, the NA carrying the genetic information all seems to have the simpler structure, with only the four, stereotyped bases.

It seems likely that DNA is required as a direct agent for protein synthesis, not only in the special case of certain viruses and bacteria but also within the nucleus of all cells (Allfrey *et al.*, 1955b; Mirsky *et al.*, 1956). The study of isolated nuclei has confirmed this, though it is not yet certain whether the DNA acts simply as a direct template (Chantrenne, 1961). As in the case of RNA, it may not be necessary that DNA should be continuously synthesized for the protein synthesis to continue. Bacteria are unsatisfactory for the study of this problem because there is no clear distinction between nuclear and cytoplasmic synthesis.

There are three protein components which might be synthesized under the control of DNA in the nucleus, the chromosomal protein, the protein of the nuclear sap, and protein for export into the cytoplasm. The chromosomal protein is mainly a basic protein, either protamine or histone, together with a small amount of "chromosin" which is richer in variety of a.a.s. The basic protein increases in parallel with the DNA (Prescott, 1961; Swift, 1962); this is not proof that the latter controls its synthesis, but it is the simplest hypothesis at present. Histone is associated also with the messenger RNA (Leslie, 1961) and, like the latter, therefore may be synthesized under the control of DNA. There is also evidence that richer proteins, containing phenylalanine, are synthesized on the chromosomal loops of DNA (Gall and Callan, 1962). Orthodox enzymes therefore may not be required and are not abundant in nuclei. There is little knowledge of the synthesis of the proteins of the nuclear sap.

It is not yet possible to add much with certainty to the picture already given (p. 205) of the way in which the two NAs operate as templates for protein synthesis, though two separate approaches have greatly strengthened the general case for one of the possible systems in which constellations of three neighbouring nucleotides in a NA molecule serve each as a code-template for one a.a. One approach is to attempt to find out which a.a.s are incorporated into peptide

linkage in the presence of particular, simple, laboratory polynucleotides (Lengyel *et al.*, 1961). The other is to study virus mutants due to particular deletions from their genome, namely the ability to synthesize particular a.a.s (Crick *et al.*, 1961). The remarkably penetrating genetical studies of Crick, Benzer and others have given very strong evidence for a trinucleotide code which is always read from a particular point, so that triplets can run consecutively throughout the genome, without either confusion due to overlapping or wastage due to the need for "punctuation marks" between them. The strongest piece of evidence is that one or two deletions, each affecting the synthesis of one a.a. result in an abnormal (mutant) type but that three deletions fairly close together can restore a virtually normal wild type virus; the explanation offered is that apart from the small region actually affected by the deletions the rest of the code can once more be read in its correct triplet sequences (Crick, 1963).

It is significant that the small damaged section of the genome should be relatively redundant. It is also interesting, but not necessarily related to this, that both approaches have given evidence of redundancy in the coding triplets, that is to say certain of the a.a.s may be encoded in more than one triplet. This may account in part for discrepancies in the codes found by different groups of workers, each having found only some of the triplets for these a.a.s. Zubay and Quastler (1962) give the following tentative codes, due to their own and three other groups. Nucleotides are represented by their initial letter, in capitals, and amino acids by their first three letters, in small type—

Authorities	Zubay and Quastler	Lengyel <i>et al.</i>	Gamow <i>et al.</i>	Woese
Amino acids				
ala	UCG	—	AAC	UAG
asp, asp·NH <sub>2</sub>	UCA	—	AGU	GAU
glu, glu·NH <sub>2</sub>	UUA	UCG	AUU, GGC	UAU
gly	UUG	UGG	CUU	GAG
leu	UCU	UUC	AGC	UCG
lys	UGA	UAA	CCC	CCG
phe	UUU	UUU	GUU	UUG
pro	UCC	UCC	CCU	CCC
ser	UGG	UUC	GGU	AAG
thr	UAG	UCC	ACU	CAC
arg	UGC	UCG	AGG	AGG
his	UGU	UAG	—	—
ileu	UAC	UUA	—	CAU
try	UAA	UGG	—	—
val	UUC	UUG	AAU	CAG
cys	?CG	UUG	—	—
met	UAU	—	—	CUU
tyr	?AU	UUG	—	UUU

Hydroxyproline is formed from proline after incorporation so that effectively all the common peptide-forming a.a.s are represented. In most cases there is no knowledge of the permutation but only of the combination of the three nucleotides, so that at best there may be a considerable measure of agreement between the various solutions, particularly in the upper sections of the table, which are best established. At the same time it is clear that there must be errors in some if there is a universal code for all organisms, which is possible, if not probable. Woese (1962) analysing the information content of the nucleotide bases decides that one base of each triplet may code by the group at position-2 in its molecule, the second by the group at position-6 and the third by the groups at the two positions in combination. On this basis he derives another set of triplets quite different from those in the table above. The results of Sueoka and Yamane (1962) and others also have their differences. The subject has reached a very challenging stage.

Both NAs have been attributed a further function in growth, that of controlling cell division, and trypaflavin, one of the inhibitors of mitosis (Table 21.2, p. 345), is a metabolic antagonist of the nucleotide bases. Certainly both forms of NA have a characteristic behaviour at the time of cell division: the chromosomes shorten, so that DNA becomes very concentrated locally, while RNA in large quantities is shed from the nucleolus on to the spindle. Ionizing rays (p. 421), and some other antimitotic agents, appear to act primarily by inhibiting DNA synthesis, and in some other instances cell division is inhibited if the normal doubling of the amount of DNA in the cell is prevented. However, in many cells DNA replication is completed long before cell division (Prescott, 1961), so that some other event must induce the latter (p. 342). Moreover, cell division can occur in the absence of the normal chromosome behaviour, and even in the complete absence of chromosomes (p. 344). There is a close correlation between the increase in DNA content of a tissue and its proliferation rate (Davidson, 1947; Hevesy, 1948), but this may be incidental: in each tissue the cell nucleus maintains a very constant average content of DNA.

There is perhaps more evidence that RNA has a specific role in cell division, as its transfer on to the spindle would imply (Swann, 1952; Mitchison, 1952; Mazia, 1956). The spindle is a cytoplasmic structure in most animal cells, and RNA is essentially the cytoplasmic form of NA. The enzyme RNAase halts cell division (Firke *et al.*, 1955; Bieseile, 1960), or causes abnormalities (Kaufman and Das, 1954), while DNAase has no effect. However, there have been other suggestions about the role of the RNA released at this time (Mitchison, 1957).

RNA is abundant in young embryos, particularly in active regions such as that of the main organizer, and it was thought to play a leading role also in the qualitative aspects of development (Brachet, 1950). However, emphasis has now shifted to the protein with which the RNA is associated (Brachet, 1960). It is claimed (De Cervalho and Rand, 1961) that the RNA from a hepatoma will induce cancer in a healthy animal whereas that from normal tissues will not, but this may be an effect purely on growth. Niu *et al.*, (1961) found that normal

RNA depressed tumour formation. In the embryo, RNA distribution in fact is related to growth activity, and only incidentally to other activities. In regeneration-blastemata growth and differentiation are rather more sharply segregated than in embryogenesis, and RNA concentration is high in the earlier, growth phase but low during subsequent differentiation (Needham, 1952, 1960a). There is little qualitative change in RNA itself during differentiation (Ebert, 1954a). As the genetic form of NA, it would be anticipated that DNA rather than RNA controls regional differentiation in the body, and there is some evidence for this (p. 391). However, it is the RNA messenger which shows the variety most clearly, because it is produced in bulk and in relatively few forms in each particular tissue.

Nucleic acids probably do perform other functions, if only indirectly through their control of the synthesis of specific proteins, but it now seems clear that the latter is their main function. They carry, and carry out, the genetical instructions about this synthesis and through the protein enzymes so formed they control the whole qualitative and quantitative pattern of biosynthesis in the cell. This means that they have some control, at least, of differentiation (p. 438) as well as of growth. In microorganisms we might logically proceed from this point directly to the genetic control of growth and development (Chapter 24). However, even in microorganisms it is profitable to consider the role of the cell organelles.

## CHAPTER 20

### *Control of Growth by Components of the Cell*

THE cell components most concerned in the control of biosynthesis are in fact those which are rich in nucleic acids, that is the chromosomes, the nucleoli, the ergastoplasmic system (Pollister, 1954; Brachet, 1957), and the supernatant cytoplasm. Two other components also are significant, the mitochondria and the Golgi system.

The general importance of the nucleus has been recognized for some years (Wilson, 1928). It is large in cancerous and other actively growing cells; a new phase of growth in the cell usually begins in the nucleus and it is here that labelled materials first collect. In erythroblasts even the iron required for haemoglobin synthesis passes first into the nucleus (Bass *et al.*, 1957) though it should be remembered that all nuclei have a rather high content of iron, and that the iron of Hb in fact is added late in the process of synthesis, out in the cytoplasm (Erikson, 1957). The nucleus is first to enlarge during the initial, lag phase of growth in the ciliate, *Tetrahymena* (Summers *et al.*, 1957), and in gland cells it enlarges at the onset of each cycle of activity (p. 119).

The enlargement is mainly related to the synthesis of the material required to "prime" subsequent synthesis in the cytoplasm, mainly the genetic messenger material, of RNA and protein, considered in the previous chapter. At the onset of a cycle of growth in many cells RNA synthesis is more rapid in the nucleus than in the cytoplasm (Brachet, 1957, p. 92); this is not evident in some cells, perhaps because they have no inactive periods (Chantrenne, 1961, p. 55). Labelled nucleotides, synthesized from labelled bases, are detected first in the nucleus and only later in the cytoplasm (Leslie, 1961). This is true also for protein (Laird and Barton, 1954): at first onset of a growth cycle the amount of protein in the nucleus first increases, and then decreases as that of the cytoplasm begins to increase. This confirms cytological evidence (Fig. 10.2) that the material synthesized in the nucleus passes out into the cytoplasm. Minot (1908, p. 185) was already aware of the shrinkage in volume of the nucleus of gland cells towards the end of a cycle of secretory activity. Isolated nuclei continue to synthesize protein for a time (Brachet, 1957), and the initial rate of this seems adequate to account for a large percentage of the total production of protein by a normal cell. In the enucleated remainder of the cell there is a rapid fall in protein content (Mazia and Prescott, 1954; Giardini, 1954). The enucleated cell of *Acetabularia* continues to synthesize protein for some time but it seems clear that the direct contribution of the nucleus is of greater importance, quite apart from its possible indirect effects on cytoplasmic synthesis. It has

been estimated (Allfrey, *et al.*, 1957) that one nucleus may synthesize 22 molecules, of molecular weight 50,000, per second, or 8 µg per hour, equivalent to its own weight every 25 minutes. This is the observed rate of growth of an active bacterium.

There is no evidence of protein moving in the opposite direction, from cytoplasm to nucleus (Brachet, 1957, pp. 108-9); in general only the small molecules of raw materials pass that way. After cell division the nuclear membrane reforms closely adherent to the chromosomes (p. 138) and so no cytoplasmic material is inadvertently enclosed. If a "hot" nucleus, labelled with radioactive material, is transplanted into a "cold" cytoplasm the latter acquires active material but there is no reciprocal movement from hot cytoplasm into a cold nucleus (p. 229).

Under these circumstances it is virtually certain that the nucleus synthesizes all of its own materials, as required. In cells which are not proliferating, however, the amount of DNA, and probably also of the other permanent constituents of the nucleus, remains very constant, with no metabolic turnover. In gland cells and oocytes, therefore, the observed activity of the nucleus is almost entirely concerned with synthesis for the cytoplasm. In proliferating cells, by contrast, each nucleus becomes two of the same size in each cell cycle. The chromosomal material is replicated *in situ*, throughout the length of the chromosomes (Allfrey *et al.*; 1957); this is now fairly certain for the DNA component, though less so for the protein. In these cells the regular replication of the chromosomes is a necessary condition for continued division, though (p. 344) it is not necessarily the only condition, or the immediate triggering agent. These aspects will be considered in the next chapter. It is evident that in such cells as oocytes and gland cells chromosomal replication is not necessary for continued production of cytoplasmic material; similarly bacteria continue to grow and so produce mycelial forms if DNA synthesis is inhibited.

Many of the lateral loops of the chromosomes, known as lampbrush loops in the oocyte (p. 124) and Balbiani rings in other cells, synthesize RNA. Each loop probably represents one gene locus and this synthesis is the gene in action. Some loops synthesize protein (Gall and Callan, 1962), or perhaps nucleoprotein. One or more special loci synthesize the RNA and protein of the nucleolus. A few of the loops synthesize DNA (Swift, 1962); the significance of this is obscure since the general view is that every locus synthesizes DNA, at the time of its own replication. This may prove to be wrong, particularly as it implies two quite distinct functions of each locus, as an autotemplate and as a heterotemplate. However, both functions have been advocated for DNAs collectively (p. 324) and they may be capable of this individually. Some loci, as their hetero function, presumably synthesize the protein of the nuclear sap, including the few enzymes which are specific to the nucleus (Hogeboom and Schneider, 1952).

The various parts of the genome are not simultaneously active in synthesis. In some, perhaps in most, tissues activity is restricted to particular Balbiani

rings. These temporal and spatial variations are to be expected, on genetic grounds. There is also evidence that synthesis may not be simultaneous throughout the length of one loop (Gall and Callan, 1962).

The nucleolus is an important organelle; it may have more than one function in growth but the main one certainly seems to be protein synthesis. There is a high correlation between nucleolar size and the protein-synthesizing activity of a cell (Sirlin, 1961), while in resting cells it is often absent (Pollister, 1954). On occasion it incorporates amino acids into proteins a hundred times as fast as the cytoplasm, and faster than other components of the nucleus (Cohen, 1959), including the chromosomes (Anfinsen, 1959). It grows faster than the nucleus as a whole (Raven, 1961). Some of the cytoplasmic protein, and the transfer (carrier) RNA of the supernatant (p. 196), and probably other constituents come from the nucleolus (Sirlin, l.c.). In the growing oocyte (p. 122), the neuron (p. 120), and other cells, nucleolar material has been seen to pass out through the nuclear membrane. In most dividing cells the remainder of the nucleolus breaks down and passes into the cytoplasm at nuclear division. It may be this which determines the sudden doubling of the growth potential at that time (Mazia, 1961). Mitchison (1957) suggests that it breaks up to form the microsomes of the daughter cells. This intermittently released component may explain why cells which are resting only temporarily during development, for instance those of the eighth thoracic segment of the isopod Crustacea, retain the large nucleolus. Enzymes such as nucleoside phosphorylases and NAD-synthetase are concentrated in the nucleolus, while alkaline phosphatase is known to pass out into the cytoplasm at the time of nuclear division.

Some of the material released from the nucleolus at this time, particularly the RNA, condenses on the spindle, and there is some evidence (Sirlin, l.c.) that its function may be to organize the spindle (p. 328). If the nucleolus is inactivated just before division is due then this is prevented (Mazia, 1961). Possibly the nucleolus also provides new chromosomal protein at this time (Stich, 1956), but there is no positive evidence that it provides chromosomal DNA at any time.

The synthesis of both RNA and protein continues in the cytoplasm. Enucleated halves of *Acetabularia* at first synthesize more RNA than nucleated halves. Synthesis continues also in enucleated amoebae, and in mammalian reticulocytes after the normal disintegration of the nucleus. In intact cells which are already in an active condition, RNA synthesis in the cytoplasm as a whole may be sixteen times that in the nucleus (Glass, 1958, p. 868), almost as rapid per unit mass as in the nucleus. Enucleated portions continue to synthesize protein for days or even weeks (Brachet, 1954; Mazia, 1956). Nucleic acid and protein extruded from the nucleus therefore probably constitute only the templates, or the enzyme systems, for the further cytoplasmic synthesis of cell-specific protein and NA. The composition of both the RNA and the protein which are active in the cytoplasm differs from that first formed in the nucleus. It has therefore been suggested that all structural protein for a particular cell

may be formed in its nucleus and that the cytoplasmic system only produces special proteins destined for secretion from the cell. In this case there should be no cytoplasmic synthesis in non-secreting cells. However, the neuron and other cells not secreting protein have in fact vigorous RNA activity in the cytoplasm, and an alternative possibility is that the fabric common to all types of cell is synthesized in the nucleus while cell-specific protein is formed in the cytoplasm. This would be plausible if cellular differentiation were restricted to the cytoplasm, but this is far from certain (p. 391). Moreover, there is definite evidence (Glass, 1958, p. 803) that proteins for the specific function of the cell are formed in the nucleus; these are probably the usual prototypes for further synthesis in the cytoplasm, and it is probable that all proteins follow this same pattern. In the cytoplasm itself there does appear to be some distinction between the synthesis of protein destined to stay in the cell and that of protein for export (p. 149), the former being synthesized on the free or postmicrosomal ribosomes (Siekevitz, 1959*b*) and the latter on the ergastoplasmic membranes, but again it is likely that the prototypes for both come from the nucleus. The difference between nuclear and microsomal RNP still needs explanation. Ribosomal RNAs vary among themselves, as might be expected if they are templates for a number of different proteins; the nuclear RNP has perhaps not yet become differentiated in this way, but this seems improbable on genetic grounds.

There is strong evidence that collectively the postmicrosomal and ergastoplasmic ribosomes control the final stages in the synthesis of all cytoplasmic proteins, and that they have the most rapid uptake of a.a.s (Brachet, 1957, p. 254; Siekevitz, 1959*b*). The fully formed proteins specific to particular cells have been found consistently associated with these granules, for instance trypsin, insulin, haemoglobin, salivary amylase and the pituitary melanophore-expanding hormone. The iron of haemoglobin is added later, however (Erikson 1957). In some cases a good proportionality has been found between the rate of synthesis of protein and the number of the particles (Loftfield, 1957; Brachet, 1957; McQuillen, 1961). The membranes themselves are less directly concerned with the actual synthesis.

The mode of action of this ribosomal system, the smallest of the structural elements of the cell, is gradually becoming clear (Roberts, 1958; Harris, 1961). There is a distinction between the relatively slow growth of the ribosomes themselves (p. 147), except after they have been depleted by starvation, and the rapid rate at which they synthesize protein products; as much as 10 per cent of their mass at any one time may be protein of this type (Siekevitz, 1959*b*), notwithstanding the rate at which it is released after completion. The slow rate of turnover of the material of the ribosomes themselves is difficult to reconcile with the brevity of life of the messenger NA (p. 326). Slow turnover would imply a catalytic function whereas a short life might be due to the messenger NA being permanently conjugated with the protein it constructs. The ribosomes are poor in orthodox enzymes and so in any case there is a strong probability that the protein synthesis is of the template type.

In *Escherichia coli* the protein of the ribosomes themselves is distinguished by the absence of cysteine and cystine, which are present in many of the product proteins, and this permits the separate measurement of the turnover of each. In passing, it is noteworthy that the difference proves, if proof were needed, that it is not the protein of the ribosomes which is the actual template for the product protein. The latter is formed from local soluble materials (p. 196); labelled a.a.s have been traced from the supernatant cytoplasm to the ribosomes and then to the supernatant once more as finished protein (Roberts, 1958). Some has been shown to pass into the smooth labyrinth of the cytoplasm (p. 146), to be further processed in the Golgi system (p. 149).

The discrepancy between the stability of the ribosomes and the lability of messenger RNA has led to the view that the latter merely becomes associated temporarily with the ribosomes (Brenner, 1962; Gros *et al.*, 1962); this might help to explain the structural differences between the RNP of the nucleus and that of the microsomal system, which may be expected to have a different history. The messenger would be the actual template and the ribosome a simple mechanical foundation.

The results of Roberts (1960) and others, however, indicate that the ribosomes are regularly recruited from the nucleus, and Britten (1962) believes that the smallest cytoplasmic RNP particle, the "cosome," is identical with messenger RNP. This grows in the cytoplasm through a quantal series of sizes to large particles, which divide and repeat the cycle. The ribosomes certainly grow and proliferate in the absence of DNA synthesis (McQuillen, 1961) but this does not rule out a vigorous recruitment from the nucleus under the influence of existing DNA. In fact, probably all cytoplasmic organelles depend on this kind of recruitment from the nucleus, if only as a method of control, and it remains possible that the active messenger template is distinct from a ribosomal template-holder.

Microsomes from liver cells, spread on the chick's chorio-allantois, appear to transfer the ability to synthesize glucose-6-phosphatase to the host's cells; those from heart cells cause sarcoma cells to produce muscle tissue (Chantrenne, 1961), so that they have considerable autonomous powers to synthesize specific proteins without continuous rebriefing from their genetically homologous nucleus.

Release of completed protein from the ribosomes is controlled by specific enzymes (Webster and Lingral, 1961), once more with the cooperation of ATP; experimentally both ATP and other pyrophosphates have been shown to dissolve out RNA from the ribosomes, together with cell-specific protein (Siekevitz, 1959b). The Mg<sup>++</sup> ion appears to act as chelating agent between protein, RNA and ATP, rather as it does between the components of the system for muscle contraction (Perry, 1956), so that once more there are resemblances between growth process and the work functions. A further resemblance is that ACh appears to induce the liberation of mature products from the ergastoplasmic template.

Acetylcholine also stimulates the turnover of phosphorus in some of the cell's P-lipids, particularly in phosphoinositides (p. 296). This may be connected with the transport of product protein through the lipoprotein membranes of the ergastoplasm, but there is an alternative possibility that it is concerned with lipid synthesis *per se*. Sterols (Kit, 1960), glycerides and other lipids are known to be synthesized on the ergoplasmic system (Brachet, 1957), probably on the lipoprotein membranes. It would therefore seem that all the main body constituents are synthesized here, glycogen (p. 325), protein, NA and lipid. The polysaccharide is probably formed on NA-free, vesicular membranes (Mercer, 1958).

As already indicated (p. 149) the Golgi system appears to be concerned specifically with the final processing of product materials for special work in the cell, or for secretion from it. The cavities of the system are probably in direct communication with those of the ergoplasmic smooth labyrinth (Fig. 11.2) and it is absent from young growing cells which lack the labyrinth. Its presence in most types of active mature cell is some further justification for distinguishing (p. 333) between the cell's specific proteins and those which it has in common with all cells and acquires in the early stages of growth, before the Golgi system appears. It may therefore be said that the system is concerned with productive and maintenance types of synthesis in adult cells, and with various work functions of the cell (p. 150).

The functions of the mitochondria in growth also have been considered already in a number of contexts, and it has become clear that they carry a battery of enzymes, mainly concerned in supplying energy for growth, as well as for other processes. In particular, by the activity of their cyclophorase system (p. 262) they couple oxidation with the phosphorylation of ADP, and this is probably why they become dispensable for some growth processes (p. 197) if external ATP is provided (Brachet, 1957, p. 278). It is probably a sufficient explanation also of the fact that the mitochondria appear to be required in order that the smaller particles may incorporate raw materials (Siekevitz, 1952). It might also be the biological reason why the mitochondria proliferate in step with the general growth of the cell (p. 145). It alone is sufficient reason why mitochondria appear at all sites of synthesis, as for instance round the nuclear membrane (p. 138). However, the mitochondria also bear enzymes directly concerned in syntheses (Schneider, 1959)—transaminases, transcarboxylases (p. 309) and others. Mitochondria promote the synthesis of hippuric acid and other pseudo-peptides, and they may be concerned with the synthesis of fatty acids and phosphatides. Their power of active locomotion in some cells enhances their value, particularly as carriers of respiratory enzymes.

The lysosomes (p. 146) also may play some part in the control of synthesis in the cell. Even if their enzymes are all lyases, working only in the direction of molecular breakdown, they may play an important part in reconstruction work, as for instance in the destruction of an enzyme when its substrate has been exhausted (p. 208) or in the more extensive reconstructions of regeneration

(von Hahn and Lehmann, 1960), metamorphosis (p. 68), and normal growth (Glucksman, 1951). Sylvén *et al.* (1959) and others believe that proteolysis plays a large part in normal growth.

The general picture of growth control in the cell therefore is that the chromosomal loci synthesize messengers which are prototypes of the templates for use in the cytoplasm and that these either become lodged on, or develop into, the ribosomal RNP particles which multiply relatively slowly in the cytoplasm. They meanwhile catalyse the much more rapid synthesis of the cell's products, particularly protein. Ribosomes for the synthesis of undifferentiated protein are free bodies while those for special proteins, whether destined for work in the cell or for export, are attached to the lipoprotein membranes of the ergastoplasm. These probably also synthesize the lipid coats for secretory granules, and lipids for other purposes, as well as other fabric materials. The microsomes from starved liver, however, inhibit lipogenesis in normal liver cells as much as 85 per cent (Massoro and Porter, 1960). This is believed to be part of a self-regulating, negative-feedback device. Special materials are further processed in the labyrinthine spaces which converge into the Golgi body. The lysosomes control any regressive processes, while the mitochondria move about supplying respiratory enzymes to liberate energy where required, and also other enzymes. Some of the a.a.s and other raw materials pass into the nucleus for the extensive syntheses initiated there, and the rest is used directly in the cytoplasm; activation systems for these raw materials occur in both parts of the cell (p. 196).

Naturally the cooperation of the various organelles in this way demands an intact cell for sustained synthesis (Hevesy, 1948; Sacks, 1953). Isolated nuclei eventually show a rapid decline in activity, just as much as the enucleated cytoplasm (Monné, 1948). Homogenates are not as active as intact cells even though the ribosomes, mitochondria and nuclei individually remain intact; clearly construction and topographical relationships also are important (J. Needham, 1936; Peters, 1937). No doubt dynamic relationships, such as the mitochondrial movements, also should be included here. This topographical aspect of organization at present seems to involve control at a higher level than that of the chemical reactions of biosynthesis. If half of the cell of the desmid, *Micraster*, lacking the nucleus is severed from the rest except for a very fine strand of cytoplasm, it nevertheless survives and grows normally. The flow of raw materials across the bridge must be limited and the implication is rather that a neurohumoral controlling agent, active in very small amounts, is responsible. This recalls such agents as the mitosis-inducing factor of *Stentor* (p. 342). At the same time the negative-feedback mechanism by the microsomes on lipid synthesis could be rather directly related to the feedback mechanisms of the enzyme level (p. 319).

It might reasonably be concluded that the cell system stands or falls as a unit, and that growth controls at the next higher level must act on the cell as an autonomous entity. There is some justification for this, as will be seen, but at the same time it is noteworthy that a number of foreign bodies are able to enter

the cell and bend its synthetic activity to their own purposes without necessarily disorganizing it completely. Examples are the viruses (p. 153), carcinogenic particles (p. 102), melanogenic systems (p. 144) and antigens (p. 214). No doubt there are other agents also which tap the cell's machinery in this way, from inside and piecemeal.

## CHAPTER 21

*Cell Division and the Control of Growth*

NORMAL growth in Metazoa, and the multiplication of Protozoa, involve a regular alternation of cell growth and cell division (p. 131), so that it would ensure coordination if one controlled the other. This is not a *sine qua non* for efficient coordination but it might reasonably be expected in the self-regulating systems characteristic of living organisms. If cell growth controls division this is of minor importance here, because the interest is primarily in the control of growth, but if cell division controls cell growth the importance is very great. Cell division is potentially a control agent as powerful as that of a switch in an electrical circuit, but it would still be important even if its control were not absolute.

In principle it is not difficult to discover if it does in fact control cell growth: mitotropic agents must be selected, that is agents which speed or retard cell division, and they must be tested for indirect effects on cell growth, through a primary action on division. It must also be possible to distinguish between these and any direct effects on growth, in parallel and not in series with the action on division. In practice this, and other, aspects of the problem present greater difficulties. The first is to recognize genuine mitotropic agents. A large number, particularly of antimitotics, have been recorded (Table 21.2, p. 345), but their number and great variety alone are grounds to suspect that many are acting on cell division only indirectly. It seems probable, in fact, that a number act primarily on cell growth, and through this on division (Mazia, 1956; Webb, 1959), so that there is great danger of pursuing the problem in circles.

Where an agent has a greater effect on division than on cell growth (Table 21.1, p. 339) there is a reasonable case for assuming a primary mitotropic effect; on this and other criteria there is good evidence for the existence of a number of natural promitotics (p. 342). Among the antimitotics, those which cause great abnormalities of the division spindle, or of the chromosomes, at the time of division seem to be genuine antimitotics. However, some of the latter group, including ionizing radiations, are suspected of acting at an earlier stage (Loveless and Revell, 1949), although the effects become visible only at division; in this event they may primarily affect chromosomal and nuclear growth, and not the division mechanism. Another group which may be primarily antimitotic are those which block or oxidize SH groups, assuming (p. 260) that a low redox potential is essential for, and specific to, the division process. The difficulty in solving this problem of the optimal redox level (p. 260) is largely bound up with the present problem of distinguishing agents primarily affecting division from

those acting initially on growth. A number of the other categories of antimitotic agent recognized in Table 21.2 (p. 346) are in effect related to one or other of the three mentioned: for instance, anticoagulants affect the spindle and probably also the chromosomes. The remaining groups, however, and many of the ostensible promitotics listed, could very well be growth agents primarily;

TABLE 21.1

**Agents Acting Differentially on Cell Growth and Cell Division****1.1. Agents which promote cell growth more than cell division, therefore producing larger cells**

Pancreas extract, and serum, on cells <i>in vitro</i> . . . . .	Willmer, 1953
Optimal conditions, for <i>Paramecium</i> . . . . .	Wichterman, 1953
Thyroid hormone . . . . .	{Hammett, 1929 Peter, 1945

**1.2. Agents which inhibit cell growth more than cell division, therefore producing smaller cells**

Inorganic salts, on <i>Tetrahymena</i> . . . . .	{Hamburger, and Zeuthen, 1957
Parasorbic acid . . . . .	Cornman, 1947
Thyroidectomy . . . . .	Hammett, 1929
Starvation, on <i>Rhodnius</i> nymphs . . . . .	Wigglesworth, 1945

**2.1. Agents which promote cell division more than cell growth, therefore producing smaller cells**

Optimal temperature (upper middle range) on <i>Chilomonas</i> . . . . .	Mucibabic, 1956
High temperature, on <i>Drosophila</i> . . . . .	Richards, 1951
Oxygen, on <i>Tetrahymena</i> . . . . .	{Pace and Ireland, 1945
Testosterone, on the rabbit . . . . .	{Montagna and Kenyon, 1949
Oestrogens, on feather growth . . . . .	Vevers, 1954
Embryo extract, on cells <i>in vitro</i> . . . . .	Willmer, 1953
Food, on pigs . . . . .	{McCance and Widdowson, 1955

**2.2. Agents which inhibit cell division more than cell growth, therefore producing larger cells**

Conditions <i>in vitro</i> . . . . .	Puck, 1957
Intermittent heat treatment . . . . .	Scherbaum, 1957
Heat, on yeasts and bacteria . . . . .	{Nickerson, 1948 Barnett, 1954
Lead nitrate . . . . .	Hammett, 1934
Nitrogen mustards, on <i>Tetrahymena</i> . . . . .	{McCance and Widdowson, 1955
Colchicine, on the rabbit . . . . .	{Haggquist and Bane, 1950
Chloramphenicol, on bacteria . . . . .	Marshak, 1955

in general, oxygen, glucose, members of the Krebs cycle, amino acids, vitamins and hormones almost certainly are. If most of the mitotropic agents listed in Table 21.2 were genuine, a number of the antimitotics should be analogues or in some other respect metabolic antagonists of the promitotics, but in fact there are few of this type, so that many must be affecting division secondarily.

Antimitotic agents are recognized first by the production of visible abnormalities at division: this clue is generally reliable but, as already indicated, not

necessarily infallible. A second criterion is the arrest of division at a characteristic stage, so that mitotic figures in that phase progressively accumulate in the tissue: this again is usually reliable but presumably not infallible. For promitotics the only general criterion is the speeding of the division process, or less reliably, the resultant speeding of the whole cell cycle; the latter is not easily distinguished from that due to a promotion of cell growth. An increase in the mitotic index, the proportion of cells in division at any moment, is subject to the same uncertainty. Moreover, an increased mitotic index could result from a retardation of the whole sequence of division, so that without further checks an antimitotic might be classified as a promitotic. Sterols in fact do retard division in this way (von Mollendorff, 1941). The difficulties in selecting genuine promitotics and antimitotics therefore is considerable. If an agent can be shown to affect only one of the components of division, the spindle, the chromosomes or the redox level, there is a strong probability that it is a genuine mitotropic but if it appears to affect all there is an equally high probability that this is secondary to an action on cell growth.

Having found reliable mitotropic agents there is the further difficulty of demonstrating effects on cell growth indirectly through division. After the application of antimitotics it may be difficult to keep tissues alive and healthy long enough to record significant sequent effects on growth. A further difficulty is to decide what kinds of sequent effects could be considered significant in the present context. Abnormalities in behaviour of the chromosomes, and possibly also of other components, during division are almost certain to affect subsequent growth of the nucleus, and so of the cell, but equally certainly serious abnormalities of the mitotic apparatus never figure in the natural controls of growth, so that the results of experiments with antimitotics must be used with caution. If cell division does normally control growth the kind of mechanism to be expected is that growth should halt at a certain critical cell size and require to be triggered off again by division. If the interval between reaching the critical size and division were a controlled variable, then the average rate of growth could be varied consequentially within very wide limits. Variations in the time spent in the process of division also could affect growth rate, but it is found that the process usually occupies a rather small fraction of the whole cell cycle, so that presumably this possibility has not been widely exploited. A further possibility is that, at the time of division, reversible physiological changes occur in the chromosomes or other components, which alter the whole tempo of growth subsequently. It might be objected that this kind of change could be effected at any stage of the cell cycle but in fact the chromosomes, at least, seem to be particularly sensitive at the time of division. Other possibilities may be envisaged but these seem the most promising bases for immediate experimental enquiry.

There are certainly agents which affect cell growth directly and not via division, and there is no question of the latter having an absolute control over cell growth. This is abundantly clear from other chapters. However, it is not

even certain that division is always a contributory controlling agent. There are a large number of antimitotics which are said not to affect growth, for instance: X-rays (Klein and Forsberg, 1954), ultraviolet rays (Cohen, 1949), colchicine (Haggquist and Bane, 1950), quinones (Bacq and Alexander, 1955,) gammexane (Lloyd, 1947), proflavin, *m*-cresol and cobalt salts (Nickerson, 1948), zinc salts (Mazia, 1956), partially oxidized sulphur compounds and lead nitrate (Hammett, 1934), penicillin (Cohen, 1949), chloramphenical (Marshak, 1955), urea (Valentine and Bradfield, 1953), folic acid analogues (Jacobson and Webb, 1952) and thyroxin (Peter, 1945). Some of these records are not in accord with evidence given elsewhere, but it seems probable that the group as a whole is a valid one (Lehmann, 1959). Equally there are agents which affect growth only, and not division.

The extensive growth of the neuron (p. 120), the oocyte (p. 122) and other postmitotic cells shows clearly that cell growth does not necessarily halt in the absence of division. Sinnott found the growth rate of apples to be very constant over a long period although the rate of cell division was changing progressively (Berrill, 1941). Gammexane arrested division completely in *Paramecium*, but the animal continued to grow and differentiate (Lloyd, 1947). By experimental means bacteria and yeasts are rather readily caused to grow as mycelial forms, growth continuing although division has been halted. Cell growth usually halts at least during the process of division (Mazia, 1956), but not always even then.

In some cases division does seem to have an effect on cell growth. As already suggested (p. 338) all agents which affect both, but division the more powerfully (Table 21.1, p. 339), may well be acting on cell growth through division, though this by no means necessarily follows. A number of reputed antimitotics do affect growth and it is probable that some, at least, are genuine mitotropics, acting on growth through this. In some cells growth ceases a considerable time before division which, therefore, may control the resumption of growth (Mazia, 1961). Perhaps the most positive evidence comes from work on *Tetrahymena* (Scherbaum, 1957); intermittent heat treatment inhibited cell division completely but growth only 35 per cent. On returning the protozoa to normal conditions 30 to 40 per cent of the cells proceeded to divide, as though further growth had been halted in just those cells which were approaching division at the time of treatment. Even this is rather circumstantial evidence, and at present it remains doubtful if cell division is one of the major factors controlling cell growth. On theoretical grounds Rashevsky (1938) regards division as a simple spontaneous consequence of cell growth. The very constant mean cell size maintained in each species of organism therefore is more probably due to this kind of control, in the reciprocal direction.

Positive evidence that cell growth and size control division was obtained by experiments on *Amoeba* (Mazia, 1956; Webb, 1959). By repeated amputation of portions of the cytoplasm it was possible to stave off division indefinitely. On the other hand once cell size was allowed to reach 80 per cent of the normal

maximum for the species division occurred, willy nilly, within 60 hours, the precise time being inversely proportional to the size achieved. This implies the production of a division-promoting substance proportional to cell mass, or to the excess above a certain threshold mass. Later work by Hirshfield *et al.*, (1960) and others, however, did not show a postponement of division in this way, by amputating cytoplasm. If the control is a customary one it is nevertheless suspended in some cases, such as that of the giant postmitotic cells already considered. Those agents (Table 21.1) which affect both cell growth and division, but the former the more strongly, may be acting on division through cell growth, but like the evidence for the reciprocal control (p. 340) this is by no means certain.

Certainly division is, to a considerable extent, independent of cell growth and size. During cleavage in the echinoderm egg the micromeres for a time divide synchronously with the larger blastomeres (Gray, 1927). In mycelial forms of bacteria unlimited growth still does not precipitate division. A discrete division-controlling mechanism has been recognized (Swann, 1954); this is preparing for the next division already during the previous one, when growth itself is at a standstill, and inhibitory agents applied at that time affect the next division whatever the intervening history of cell growth. A factor specifically promoting mitosis has been identified in plants (Miller *et al.*, 1955) this consists of "kinetin," 6-furfural amino purine, associated with the common auxin, indole-acetic acid, and it accelerates the onset of prophase. An agent promoting division diffuses from a dividing *Stentor* to a second individual to which it is parabiotically united (Weisz, 1956). *In vitro* food material directly or indirectly produces a specific division-inducing agent (Willmer, 1958); after three hours of feeding, but not less, the cell has become triggered to divide in 7 hours time irrespective of the further presence or absence of food. In this case therefore a major growth-promoting factor also induces cell division, but independently, and not sequentially. This is one aspect of multiple assurance (p. 343), namely, that if an agent acts on the sequential events of a process it does so on each individually and directly, and not simply by deputation through the sequence itself. The determination of postmitotic cells not to divide again also occurs within 4 hours of the last division (Mazia, 1960).

The conclusion seems to be that each of the two main components of the cell cycle may have some degree of control over the other and particularly growth over division, but neither is the sole control, both are in abeyance in certain natural situations, and both can be eliminated experimentally. The two components are normally integrated but the gearing is very flexible, as in so many biological mechanisms. In the proliferation cycle of a population of microorganisms cell size changes continuously (p. 114), reflecting successive differential changes in rates of cell growth and division. The former reaches a peak later in the history of the culture than cell division rate. In some bacteria (Knaysi, 1951) the rate of division later overtakes the growth rate once more; cell size therefore at first decreases, then increases, and finally decreases again.

Testosterone causes proliferation with decrease in cell size (Montagna and Kenyon, 1959) and changes in pressure and in temperature commonly affect the two components differentially. Some other agents acting differentially are given in Table 21.1 (p. 339).

The positive, but very flexible, gearing between the two is shown by the return of cell size to its initial value, at the end of a cycle of proliferation in a population of microorganisms, and to the value characteristic of the species, at the end of the cleavage phase of development of the zygote. It is shown also by the accelerated rate of division of protozoa in which division has previously been differentially inhibited by heat treatment (Zeuthen and Scherbaum, 1954; Scherbaum, 1957). In developing trout, and in the metamorphosis of insects (p. 76) some large postmitotic cells resume division and return to the normal size of diploid cells. Mazia (1961) decides that cell growth controls cell division in the long run but not necessarily through a precise maximal size in each cycle.

The degree of independence between the two components is probably another manifestation of multiple assurance, a phenomenon of great importance in embryogenesis (Huxley and De Beer, 1934). Its main virtue is in preventing any component of the process from being too vulnerable to fortuitous errors in any one controlling factor. The flexibility which results is a conspicuous feature of all the components of the cell cycle, and merits further consideration at this point. Similar partial independencies between components are seen at other levels of the growth process (J. Needham, 1942).

The growth of the nucleus is a major factor in the control of the whole cell cycle (p. 330). It controls cytoplasmic synthesis (p. 330) and is the first evident response of the cells of *Aedes* to food (Wigglesworth, 1942). It is completed early in the cell cycle in the epidermis of the axolotl (Overton, 1955). Rapidly proliferating cells (Green *et al.*, 1959), including embryonic and neoplastic cells, have a high ratio of nuclear to cytoplasmic size (N/C), and in a population of micro-organisms this ratio is maximal when proliferation rate is maximal. The absolute size is sometimes low, owing to rapid division, but it is clear that nuclear growth improves its lead over cytoplasmic growth, under these conditions. The N/C ratio in polyploid and polytene cells is usually fairly similar to that of normal diploid cells of the species so that here nuclear growth and size probably control those of the cytoplasm. Alternatively, or in addition, they possibly control cell division, since this occurs at a proportionately greater size in the polyploid; if so then this is an inhibitory type of control, that is to say the larger nucleus delays division until the cell is proportionately larger than that of the diploid. The onset of cell division is usually closely correlated with a critical nuclear size (Salvatore, 1950).

Nuclear growth is completed early in the cycle of rapidly proliferating cells, but progressively later in more slowly growing cells (Ris, 1955). By an extension of this trend there should result very slowly growing cells which never divide, have a relatively small nucleus and reach a giant cytoplasmic size; in fact this seems to be essentially the condition in typical postmitotic cells. The

mechanism seems to show an analogy with the response of excitable tissues to an electrical stimulus: if the current strength rises slowly the threshold for response by the tissue may rise disproportionately, and there comes a stage where the stimulus may fail to excite, however powerful.

It is clear from this that the control of the cell cycle by nuclear growth again is not absolute. Indeed in syncytial structures nuclear growth and division may continue indefinitely without cell division, though cytoplasmic growth is proportional. In some bacteria the nucleus regularly divides half a cell cycle ahead of the cytoplasm (Maaloe and Lark, 1954). Reciprocally enucleated cells sometimes divide (Mazia, 1956). Moreover, nuclear growth to some extent is independent of nuclear division; thyroxin and *p*-aminobenzoic acid can produce giant nuclei by promoting their growth without division (Peter, 1945; Thomas, 1942). Conklin found that the nucleus reached a variable size, depending on the duration of the interphase (Robertson, 1923, p. 155). In these cases the growth of the nucleus does not even determine its own division. In syncytia all the nuclei divide synchronously and therefore presumably are controlled by the cytoplasm (Mazia, 1960). Abundant food favours a relatively large nucleus.

Lorch (1952), in particular, has cited many further examples of relative independence between components of the cell's cycle of growth and division, mainly concerned with the latter. The existence of polyplloid and polytene nuclei shows that chromosomal and chromatidal division can occur without nuclear division; as already noted, nuclear division can on occasion overtake this once more, and restore the diploid condition. Nucleus and cell may still divide after removal of the centrosome, a new one sometimes being regenerated, but not always. Asters isolated from the cell can continue their development. *In situ* the centrosome can pass through successive generations of division, with aster formation, in the absence of the nucleus; the cytoplasm may undergo partial clearage under these conditions. In fact, the cytoplasm can divide in the absence of both nucleus and centrosphere. In the absence of centrospheres and of nuclear division, cycles of nuclear membrane breakdown and reformation (p. 138) can continue. Regular cycles of change in the cortex of the cell may keep pace with those of the chromosomes even when the development of the spindle has been inhibited with colchicine. Finally a cycle of change in oxygen consumption may persist when all other evident signs of the cell cycle have been abolished (Zeuthen, 1951; Swann, 1952). Among the components of cell growth, nitrogen fixation can be partially independent of cell division (J. Needham, 1942, p. 515), volume increase can be relatively independent of weight, protein synthesis of RNA synthesis (Mazia, 1961) and of weight (p. 118), water of other constituents, and so on.

A particularly interesting example, because a slight differential shift in timing between the two can have such a dramatic effect, was noticed in the early cleavage of the echinoderm egg (Horstadius, 1928). There is a regular 90° change in orientation of the division spindle in successive cleavage generations

and by experimental means this change may be shifted relatively to the time of onset of the cell divisions so that the spindle orientation is overtaken in a 45° position to its successive normal orientations. The resulting pattern is essentially similar to that which has become normal in those invertebrates having spiral cleavage.

The extent of this loose articulation between the components certainly adds to the difficulty in solving the problem of cell division and the control of growth. Equally, however, it adds to the importance of solving the problem in detail, the ultimate aim being a quantitative measure of the various partial dependencies. The clarification of the picture concerning mitotropic agents (Table 21.2) is the most important of the more immediate aims, which still centre on the two main components of the cell cycle. Meanwhile it will remain reasonable to use the rate of cell division as an approximate index of growth rate, on the assumption that there is usually a high degree of correlation between the two.

TABLE 21.2  
Mitotropic Agents

1. Promitotic agents

1.1. Physical factors

Temperature increase . . . . .	. . . . .	{see Hughes, 1952a Mučibabić, 1956
Mechanical stretching . . . . .	. . . . .	Wigglesworth, 1945
Hydrostatic pressure . . . . .	. . . . .	see Hughes, 1952b
Mechanical irritants . . . . .	. . . . .	see Fischer, 1946
Low dosage of ionizing radiations . . . . .	. . . . .	see Hughes, 1952a
Hypotonic conditions . . . . .	. . . . .	see Hughes, 1952a
Increased hydration of proteins . . . . .	. . . . .	Lasnitski, 1945

1.2. Chemical agents

Salts (KBr, K <sub>2</sub> SO <sub>4</sub> ), alkalis (KOH, NaOH)	. . . . .	see J. Needham, 1931, p. 538
Zn <sup>++</sup> . . . . .	. . . . .	see Mazia, 1956
Cyanate (repeated administration) . . . . .	. . . . .	Dean and Gunz, 1950
Ethyl urethane . . . . .	. . . . .	Mookerjee and Datta, 1959
Alcohol . . . . .	. . . . .	see J. Needham, 1931
Croton oil . . . . .	. . . . .	see Bullough, 1952
Glucose, glycogen . . . . .	. . . . .	see Bullough, 1952
Succinate, fumarate . . . . .	. . . . .	see Brachet, 1957
Glutamic acid . . . . .	. . . . .	Bullough and Johnson, 1951
Tryptophan . . . . .	. . . . .	see Wardlaw, 1952
Cysteine . . . . .	. . . . .	Hammett, 1934
Glutathione (GSH) . . . . .	. . . . .	see Stern, 1956
Peptides . . . . .	. . . . .	see Hughes, 1952a
Thrombin . . . . .	. . . . .	Fischer, 1936
Embryo extract . . . . .	. . . . .	see Willmer, 1953
Ribonuclease (small amounts) . . . . .	. . . . .	Kaufman and Das, 1954

1.2.1. Vitamins and related substances

Biotin . . . . .	. . . . .	Wilson and Leduc, 1949
Mesoinositol (myoinositol) . . . . .	. . . . .	Chargaff <i>et al.</i> , 1948
Pteroyl glutamic acid (PGA) = folic acid . . . . .	. . . . .	see Jacobson and Webb, 1952

TABLE 21.2—(cont.)

Xanthopterin . . . . .	see Willmer, 1953
"Vitamin B <sub>14</sub> " . . . . .	Norris and Majnarich, 1949
Adenosine triphosphate (ATP) . . . . .	Barnett, 1954
1.2.2. <i>Hormones and related substances</i>	
Anterior pituitary growth hormone (APGH) . . . . .	see Hanstrom, 1939
Posterior pituitary hormones . . . . .	Bramstedt, 1937
Thyroid hormone . . . . .	Smith, 1951
Adrenal cortical hormones . . . . .	see Hanstrom, 1939
Adrenalin, pilocarpine . . . . .	see J. Needham, 1931
Androgens, oestrogens . . . . .	{ Montagna and Kenyon, 1949 see Bullough, 1952
Crustacean eye-stalk hormone . . . . .	Kropp and Crozier, 1934
Insect prothoracic hormone . . . . .	see O'Farrell and Stock, 1954
Auxins . . . . .	see Hanstrom, 1939
Kinetin . . . . .	Miller <i>et al.</i> , 1955
1.3. <i>Physiological factors</i>	
Food . . . . .	{ Day, 1949 McCance and Widdowson, 1955
Rest . . . . .	Prescott, 1955
Wounding . . . . .	{ Bullough, 1948 see Fischer, 1946
	{ Overton, 1955
2. <i>Antimitotic agents</i>	
2.1. <i>Physical factors</i>	
Mechanical shaking . . . . .	see Heilbrunn, 1952
High hydrostatic pressure . . . . .	Harris, 1948
High temperature . . . . .	{ see Hughes, 1952a Zeuthen and Scherbaum, 1954
Low temperature . . . . .	see Heilbrunn, 1952
Light . . . . .	Morton <i>et al.</i> , 1940
Electric shock . . . . .	Holzbauer <i>et al.</i> , 1952
Ionizing radiations . . . . .	numerous
Hypertonic conditions . . . . .	see Hughes, 1952a
2.2. <i>Chemical agents</i>	
(The reference cited is not necessarily responsible for both the evidence of antimitotic action and the interpretation of the mode of action)	
2.2.1. <i>Substances affecting respiration</i>	
N <sub>2</sub> , N <sub>2</sub> O, argon, propane . . . . .	Fergusson <i>et al.</i> , 1950
CO <sub>2</sub> . . . . .	Voegtlind, 1934
Lactic acid, pyruvic acid . . . . .	Voegtlind, 1934
Sodium fluoride . . . . .	O'Connor, 1950
D-glucosamine . . . . .	Quastel and Cantero, 1953
Malonates . . . . .	{ see Swann, 1954 Brachet, 1957
Urethane, phenylurethane . . . . .	see Danielli, 1951
Bromine . . . . .	Chury and Slouka, 1949
CN, azide, CO . . . . .	see Swann, 1954

TABLE 21.2—(cont.)

2.2.1.1.	<i>Anti-SH agents</i>	
	Iodoacetic ester, maleic acid, mercurials, arsenoxides, benzoquinone, quinonimines, cacodylates, coumarins, para-sorbic acid and other unsaturated lactones	} see Loveless and Revell, 1949
	H <sub>2</sub> S, mercaptans, thiols, and other analogues	} Voegtlín, 1934
	Partially oxidized S-compounds	} Hammett, 1934
	Iodoacetate	} O'Connor, 1950
	Lachrymator gases, chloracetophenone	} (see Dixon, 1948)
	Ionizing radiations, radiomimetic substances	} (see Hughes, 1952a)
		Bacq and Alexander, 1955
2.2.1.2.	<i>Agents affecting phosphate metabolism</i>	
	Beryllium salts	{ Chèvremont and Firket, 1951 (see Brachet, 1957)
	Phlorrhizin	Bullough, 1949
	Dinitrophenol } (uncoupling agents) Thyroxin, usnic acid	see J. Needham, 1942, p. 570
2.2.1.3.	<i>Narcotics</i>	
	Urethane, phenylurethane	see under 2.2.1 (above)
	Ether	see Swann, 1954
	Chloroform	see Hughes, 1952a
	Narcotine	Lettré and Albrecht, 1942
2.2.2.	<i>Lipids</i>	
	Glycerides	{ Levan and Ostergren, 1943 (Gavaudan <i>et al.</i> , 1943)
	Sterols	von Mollendorff, 1941
	Stilboestrol	Lehmann <i>et al.</i> , 1945
	Benzene	see Hughes, 1952a
2.2.3.	<i>Carcinogens or co-carcinogens</i>	
	20-methylcholanthrene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene	} Demerec, 1947 (see Heilbrunn, 1952)
	Sterols	Hieger, 1957
	Croton oil	Setala, 1954
	Cobalt	Nickerson, 1948   Heath, 1954
2.2.3.1.	<i>Anticarcinogens</i>	
	Maleic acid	Crabtree, 1945
	Heparin, Shear's polysaccharide	Heilbrunn, 1956
	Dinitrophenol, bromobenzene } Quinones,	see under 2.2.1.2 (above)
	aminopterin (folic acid antagonists)	Boyland, 1949
2.2.4.	<i>Agents affecting the division-spindle (C-mitotic agents)</i>	
	Colchicine	Levan, 1938
	Coumarin	Cornman, 1947
	Emetine, berberine, narcotine, cacodylates	Lettré and Albrecht, 1942
	Ether	see Swann, 1954

TABLE 21.2—(cont.)

Chloroform . . . . .	<i>see Hughes, 1952a</i>
Halogenated hydrocarbons . . . . .	<i>see Sexton, 1953</i>
Acenaphthene . . . . .	<i>see Loveless and Revell, 1949</i>
Camphor, butyl alcohol, benzene . . . . .	Levan, 1946
Hydroquinone . . . . .	<i>see Ris, 1955</i>
N <sub>2</sub> , N <sub>2</sub> O, argon, propane . . . . .	Fergusson <i>et al.</i> , 1950
Aminopterin and other folic acid analogues . . . . .	<i>see Hughes, 1952b</i>
Ribonuclease . . . . .	<i>see Brachet, 1957</i>
Heparin . . . . .	{Fischer, 1936 Heilbrunn and Wilson, 1949}
Janus green B . . . . .	Hughes, 1952a
<b>2.2.4.1. Anticoagulants</b>	
Heparin, Janus green B . . . . .	<i>see under 2.2.4 (above)</i>
Shear's polysaccharide . . . . .	Kreisler, 1952
Colchicine, quinones . . . . .	<i>see Ris, 1955</i>
Citrate, oxalate (Ca-precipitants) . . . . .	<i>see Heilbrunn, 1952</i>
Hypotonic media, low hydrostatic pressure . . . . .	<i>see Ris, 1955</i>
<b>2.2.4.2. Protein-denaturing agents</b>	
Lead nitrate . . . . .	Hammett, 1934
Urea (H-bond breaking) . . . . .	{Wilson, 1906 Valentine and Bradfield, 1953}
X-rays . . . . .	{Sparrow <i>et al.</i> , 1952 Rozendeel <i>et al.</i> , 1951}
<b>2.2.5. Nucleotropic Agents</b>	
<b>2.2.5.1. Agents acting on the resting nucleus, causing fragmentation of chromosomes at the next division (Radiomimetic agents: Loveless and Revell, 1949)</b>	
N- and S-mustards . . . . .	{see McCance and Widdowson, 1955}
Diepoxides, protanemonin, divinyl sulphone, aminostilbenes, carcinogenic hydrocarbons, urethane, caffeine . . . . .	Loveless and Revell, 1949
Gammexane (hexachlorocyclohexane) . . . . .	{Lloyd, 1947 Kostoff, 1948}
Folic acid analogues . . . . .	Dustin, 1950
<b>2.2.5.2. Agents acting on the dividing nucleus, causing pycnosis, and clumping of chromosomes</b>	
Trypaflavin, acridines, aminopterin . . . . .	{Dustin, 1947 O'Connor, 1949}
Caffeine . . . . .	Kihlman and Levan, 1949
Sodium ribonucleate . . . . .	Ojima and Ogaki, 1950
Iodoacetic ester, fluorides, maleic acid . . . . .	{Loveless and Revell, 1949 O'Connor, 1950}
Arsenoxides, urethane . . . . .	Loveless and Revell, 1949
Cyanates . . . . .	Dean and Gunz, 1950
Organic mercury compounds . . . . .	Dustin, 1947
Quinones . . . . .	Parmentier and Dustin, 1948
Quinonimines, di-imines, mono- and poly-phenols, cacodylates, coumarin . . . . .	Loveless and Revell, 1949

TABLE 21.2—(cont.)

2.2.5.3.	<i>Nucleic acids, derivatives, analogues, antagonists, etc.</i>	
	RNA and DNA in high concentration (2 per cent)	Mittler and Herman, 1950
	Sodium ribonucleate . . . . .	Ojima and Ogaki, 1950
	ATP, AMP . . . . .	Bullough and Green, 1949
	Guanylic and cytidylic acids . . . . .	see Hughes, 1952a
	Adenine, diaminopurines . . . . .	see Hughes, 1952b
	N-mustards (react with PO <sub>4</sub> groups) . . . . .	Elmore <i>et al.</i> , 1949
2.2.6.	<i>Vitamins and analogues</i>	
	Ascorbic acid (vitamin C) . . . . .	Shapiro, 1948
	p-Aminobenzenesulphonamide . . . . .	Thomas, 1942
	Aminopterins . . . . .	Dustin, 1950 Lutwak-Mann, 1952 see Ris, 1955
	Menadione (2-methyl-1,4-naphthoquinone) . . . . .	Lehmann <i>et al.</i> , 1945
2.2.7.	<i>Hormones and analogues</i>	
	Thyroid hormone . . . . .	Peter, 1945 Mittler and Hermann, 1950
	Adrenocorticotropic hormone, gluco- corticoids . . . . .	see Bullough, 1952
	Steroid hormones in general . . . . .	von Mollendorff, 1941
	Stilboestrol . . . . .	Lehmann <i>et al.</i> , 1945
	Adrenalin, sympathomimetic substances including colchicine . . . . .	Lettré and Fernholz, 1943 see Bullough, 1952
2.3.	<i>Physiological agents</i>	
	Inhibitory factor of serum . . . . .	Müller, 1956
	Work-functions . . . . .	Blumenfeld, 1944
	Shock . . . . .	Bullough and Green, 1949

## CHAPTER 22

### *Control of Growth at Tissue and Organ Levels*

THE controlling agents so far considered act within the growing cell, directly on the reactions of biosynthesis or in some cases, perhaps, on the motor process of cell division. Between this level and that of systemically acting agents there are local controls acting between cells, tissues and organs. These play an important part in the more detailed determination of harmonious growth between parts, especially functionally related parts. The most common of these controls are by direct contacts between neighbouring tissues, but a number of agents which are potentially systemic agents, because they are systemically distributed, in practice act very locally: examples are the nervous system, a set of blood-borne, organ-specific, controlling agents (Weiss, 1955), and also to some extent the true hormones, since they often have actions limited to particular target organs. However, in general the hormones have widespread effects and will be considered in the next chapter.

#### **22.1. Direct Contact Agents**

Tissues are sensitive not only to neighbouring tissues but also to local mechanical and other stimuli from foreign bodies, which in some cases throw light on the more physiological interactions. Beads and other inert bodies will induce proliferation in the mammalian uterus (Wyburn-Mason, 1950, p. 246), and the growth of bones is affected by experimentally applied local forces (Murray, 1936; Le Gros Clark, 1958). Here growth is orientated so as to strengthen the bone against the imposed forces. Both this and the uterine response are models for natural responses. In the insect, *Rhodnius*, distension of the body by food or by mechanical means induces epidermal proliferation and the development of new sensory plaques (Wigglesworth, 1945; 1948b). There is a time-lag in the response, so that new plaques do not appear until the second moult after distension. This can be a general response affecting the whole body, but it is localized after injury and the subsequent local tensions of healing. Local oxygen shortage, produced by implanting a crystal of pyrogallol, will induce a local proliferation of the tracheal respiratory tubes of insects (Wigglesworth, 1954a). This response is interesting also because it is accompanied by a movement of neighbouring tracheoles towards the area, a physiological response to supplement the morphogenetic one. Local illumination can cause a local production of new melanophores, as well as an increase in the amount of melanin in existing cells (Fox, 1953, p. 229), and exclusion of light leads to the reversed, atrophic response. Here again, there is usually also a physiological response,

that of pigment dispersion or retraction. This is essentially a case of functional allotropy, which in general is a local response, and it indicates that the same factor which induces the function may also induce the hypertrophy. If this is generally true there may be a very large number of varied factors controlling growth at this level, throughout the body.

Functional hypertrophy responses of this kind act as a reminder that normal maintenance, the replacement of material used up in the work functions, comes into the same category, essentially local in causation and manifestation. The taking of food by the snail *Helix* and the crayfish *Astacus* induces the secretion of digestive enzymes and this in turn leads to renewed cell proliferation in the digestive glands (Krijgsman, 1925, 1928; Hirsch and Jacobs, 1928, 1930). The precise mode of intermediation of this response is still uncertain; probably each of the many other instances of the phenomenon must be considered as a separate problem. It is a subject which already could be reviewed with profit. Wounding the lens is known to induce DNA synthesis after a lag of 14–16 hours (Harding and Srinivasan, 1960).

There is some interaction even between cells of the same tissue. Nuclear and cell division are sometimes synchronized throughout a tissue (Dixon, 1946), just as nuclear division is, in some syncytia. The synchronization has been attributed to the diffusion of a mitotic hormone, though it is not clear where this is first produced or how it diffuses so rapidly. There may be open connexions between the cells of many tissues. Experimental methods of inducing synchronization in a population of cells (p. 341) indicate that external factors conceivably might be responsible here, also. Once established, synchronization might persist automatically in a pure line of cells. In this event there would not necessarily be any interaction between the family of cells. Cell-to-cell interaction is seen between nerve cells, but it is more convenient to consider this below.

The action of one tissue on its neighbour may stimulate or inhibit its growth, as would be expected if this plays a part in establishing and maintaining normal proportions among tissues and organs. Odontoblasts stimulate the activity of the epidermal ganoblast layer of teeth, and reciprocally, the epidermal hair follicle stimulates the dermal papilla of the hair. The latter may exert an inhibitory control over the epidermal component, since this tends to become cancerous in its absence (Wolbach, 1950). Thus the combination is a self-regulating system, analogous to that of the pituitary and the other endocrine organs, at another level (p. 360). It appears that the dermal papilla may also have some power of positive induction of hairs, in regenerating skin (Billingham, 1958). Some cases of local inhibition may be due simply to competition for nutrients, as when a wave of hair growth in rodents suppresses the growth of epidermal warts (Mottram, 1945).

For the growth of the chick's wing mutual stimulation between ectoderm and mesoderm is essential (Hadorn, 1961). The epithelia of skin, vagina, uterus and other organs do not respond to oestrogens unless they are in contact with

connective tissues (Lasnitski, 1958). The stroma of an organ with carcinomatous epithelium hypertrophies in step with the latter, though without necessarily becoming cancerous itself (Reimann, 1947); after the malignant tissue is destroyed the stroma spontaneously regresses. This is probably a relatively non-specific effect on any tissue which chances to be near the cancerous cells, but in general these local actions are probably specific. This is the impression also from the instances studied *in vitro* (p. 91; Fischer, 1946). The response depends quite as much on the recipient as on the donor tissue. For instance cell proliferation in the lens of a vertebrate embryo is not at the surface of contact with the stimulating eye cup. The special case of nerve fibres and their end organs is considered in the next section.

Among the most interesting of experimental cases are those in which part of the amphibian eye, the lens or the eye cup, is grafted on to the other part *in situ*, in individuals of different age or of different species. The growth of both parts is changed, the one speeded and the other slowed, so as to give a well-proportioned eye (Huxley, 1932, p. 195; Twitty, 1955). The extrinsic eye muscles adjust similarly (Twitty, l.c.). By contrast whole eyes grafted in this way tend to grow at their intrinsic rate and only later does the host induce some degree of size regulation. In this case there would be a less intimate topographical relationship between graft and host tissue and the implication is that local controls can be more potent than systemic ones. Growing vertebrates are already gross mosaics of independent organ systems at a time when there is still regulation on a smaller scale between tissues and parts of organs, and the tissue interactions under consideration are probably relics of the mosaic progression of early ontogenesis. Initially the major features of development are controlled by these local contacts. In the eye-grafting experiments there is probably evidence for the coexistence of these local controls at the tissue level alongside the vascular and hormonal controls subsequently established at the systemic level. The interaction between tissues during embryogenesis is largely competitive. Thus, in the *notch* mutant of *Drosophila* the nervous system hypertrophies enormously at the expense of the general ectoderm. There are many other cases of this kind of abnormal development (Hadorn, 1961, p. 302).

## 22.2. Control of Growth by the Nervous System

The growth and subsequent maintenance of each organ depends to a considerable extent on the peripheral nerves supplying it (Wyburn-Mason, 1950). If a growing limb is denervated its growth is seriously retarded, particularly if re-innervation is prevented (Huxley and De Beer, 1934, p. 430; Murray, 1936). Reciprocally an augmentation of the normal nerve supply to a limb may cause great hypertrophy or the production of supernumerary limbs (Huxley and De Beer, l.c., p. 230). Apparent exceptions are the growth of the antlers of the stag (Wislocki, 1956) and of hair in mice (Haddow *et al.*, 1945): in both cases no positive effect of the peripheral nerves has been detected.

This neural tropic effect on growth should be distinguished from functional

hypertrophy, the further auxotropic effect of using the organ, though conceivably the nerve supply may play a part in this also. It is possible that the amount of the auxotropic factor produced by a nerve is proportional to its own functional activity, since it is known that the nerve cell enlarges as a result of activity (Chance, 1956), and that the magnitude of the resting potential is related to that of the previous action potential (Grundfest, 1955). However, it is also clear that the auxotropic factor is already being produced by nerves long before they become functional in impulse conduction; functional hypertrophy and disuse atrophy therefore may involve merely quantitative changes in the production of an agent present from the outset. The production of this may be stimulated by nervous activity but is not necessarily itself a component of the mechanism for impulse conduction. There is the alternative possibility that hypertrophy and atrophy depend more on the end organs themselves than on the nerve, and this would be a rational biological adaptation. A more positive reason for supporting this view is that there is considerable disuse atrophy in an immobilized limb, although its nerves may be little inactivated because they are so extensively integrated into the neurogenic pattern of the whole set of limbs, and the motor nerves remain continuously stimulated via the C.N.S. (Gray and Lissmann, 1946, 1947). When limbs are also de-efferentiated disuse atrophy is necessarily supplemented by denervation atrophy, but the two should not be confused.

The tropic effect of the peripheral nerve on growth is seen also in regenerating limbs (Singer, 1952, 1959, 1960), so much so that the limbs of adult Anura, which do not normally regenerate, will do so if their nerve supply is augmented. The quality of the nerves, sensory or motor, seems immaterial here; the effect is simply quantitative, and non-specific, and it flows both ortho- and antidiromically. It requires no appreciable influence from neurons other than those immediately supplying the limb (Needham, 1953), and this is true also for the influence during embryogenesis. If regenerating limbs are denervated their growth ceases until they become re-innervated. By contrast the neural influence is not required for their differentiation.

The growth of nerve tissue itself, during embryogenesis, depends on a similar tropic influence, from other parts of the nervous system, and ultimately, perhaps, from the initial organizer of the embryo. There is also the corresponding maintenance influence in the mature nervous system; if a pathway is interrupted at any point there is eventually transneuronal degeneration in other cells of that pathway. The influence begins on the afferent side and flows progressively downstream (Huxley and De Beer, l.c., p. 385; Weiss, 1955) to the motor end. The dorsal root cells derived from the neural crest therefore play a leading role in the whole mechanism, in vertebrates (Hörstadius, 1949).

Like the action of nerve fibres on other tissues this influence on the growth of nerve cells will also pass antidiromically, for instance from a muscle, to affect the size of the lower motor nucleus (Weiss, 1955), and from a sensory nerve to its sense cell. Effector organs other than muscles also possibly have this effect,

since extracts of salivary glands are among the most potent (Cohen and Levi-Montalcini, 1958). *In vitro* these promote both the proliferation of nerve cells and the sprouting of their fibres. The property may be even more widespread since extracts of cancerous tissues are very potent. There are three groups of effect, therefore; nerve tissue on other tissues, other tissues on nerve tissues and homotropic effects by one nerve cell on another.

In connexion with the neurotropic action on tissues generally, it may be significant that the  $\alpha$ -rhythm of the cerebral cortex changes with age in a way which parallels the change in general growth rate (Weinbach, 1938). Schneider (1944) explored the consequences of assuming that growth could be treated as an electro-chemical process (cf. p. 409), in which the C.N.S. provided the driving force, so that its mass could be used as a measure of that force at any moment. The consequences were consistent with other facts about growth and seemed to justify the general assumption. At the same time it is necessary to remember that the growth of any organ, including the C.N.S., *must* show a general correlation with that of the rest of the body, without necessarily being a causal agent of the latter. Perhaps more impressive in the present context is the fact that throughout development, including the apparent chaos of metamorphosis (p. 68) the nervous system of an insect grows perfectly steadily, alone among the organs (Power, 1952); it is tempting to assume that whatever the other organs may do the nervous system executes a precise, uninterrupted growth programme which it imposes on them all, in due course. It is therefore possible that the effect of nerve tissue on growth is not purely local and that through the organization of the C.N.S. these local actions are all integrated.

Naturally the question of primary interest concerns the nature of the neurotropic influence. In general it is more probable that a chemical substance is involved than an electrochemical mechanism, just as in the case of impulse transmission at synapses. It seems reasonable to believe that the agent does act through these synapses, though in fact Overton (1950) found that grafted nerve tissue stimulated growth in neighbouring host tissue without forming synapses; moreover, effective material is extracted in quantity from nerve tissue (Hughes, 1952a; Willmer, 1958), implying that it is not restricted to the synaptic region. It was natural to expect that the synaptic transmitter of impulses itself might be the auxotropic agent: this would be a sound piece of economy and there is considerable evidence in its favour. It is known that ACh is released continuously at synapses in small quantities, even in resting systems (Katz, 1959) and this could control steady growth and maintenance while the extra ACh liberated upon impulses transmission might control functional hypertrophy. In the arthropods, particularly, neurosecretory cells play a great part in systemic growth control, and it is reasonable *a priori* presumption that the secretion is a specialization of synaptic secretion. A great deal of evidence in favour of the general view was collected by Wyburn-Mason (1950), and this was supported by work on regeneration (Singer, 1952; 1959; Needham, 1960a). Acetylcholine and other parasympathetic agents proved generally stimulatory (Wyburn-

Mason, I.c.), whereas sympathetic and related agents, epinephrine, adrenaline, colchicine and others inhibit cell division (Lettré and Fernholz, 1943). On organs with the dual autonomic innervation, therefore, there might be a two-way control of growth. The physiological change of colour in some animals is controlled in such a closely similar way to the morphogenetic production of new pigment that identical mediators could reasonably be expected. Again the movements of  $\text{Na}^+$  and  $\text{K}^+$  ions across the nerve-cell membrane during the impulse conduction and recovery show a close parallel to the respective movements during wounding and subsequent healing of tissues (p. 291) (Needham, 1960a). Nerve impulses, like the auxotropic action, pass anti- as well as orthodromically, though unlike the latter they do not pass synapses antidromically. Hyperalgesia is believed to be associated with antidromic phenomena and there is often tissue hypertrophy in hyperalgesic regions (Lewis, 1943). "Royal jelly," which stimulates the growth of female bee larvae so that they become queens, contains a large amount of ACh, six times as much as the brain (Colhoun and Smith, 1960).

At the same time there is considerable evidence against the identity of transmitter and auxotropic agents, however, and this has been strengthened by work in the field of regeneration (Singer, 1960), which previously seemed to offer most support for the identity. As already noted the auxotropic effect is active before ACh can be demonstrated in embryogenesis. An anaesthetized nerve, incapable of conducting impulses, has been found still to promote growth, though it could be argued that the resting output of ACh would continue under anaesthesia. Lender (1952) found that a factor from the brain of planarian worms, which induces the regeneration of eyes, does not travel via the optic nerve at all. Singer's critical discovery is that sensory nerves, which produce very little ACh at their peripheral endings, have an auxotropic effect as powerful as motor nerves, which produce much ACh. In spite of the correlation between ACh content, cholinesterase activity and growth in regenerating structures, therefore (Singer, 1960), the auxotropic agent may not be ACh. Conceivably it may be one of the less well-known transmitter agents such as  $\delta$ -hydroxytryptamine or  $\gamma$ -aminobutyric acid. It may even prove to be one of the growth hormones, particularly in arthropods where these are so extensively of neural origin.

Direct attempts to isolate the active principle from nerve tissues are still in their early stages. Overton (1955) found it soluble in water and relatively resistant to alcohol, ether and cold. It is a small molecule diffusing at the relatively rapid rate of 2 cm per day, which is comparable to the rate of flow of the axoplasm itself (p. 120). Lender's *organisine* in planarians differs in properties (Needham, 1960a), but this may be a differentiation factor rather than the growth agent: the auxotropic agent has no effect on differentiation (Singer, 1952). Koechlin and von Muralt (1946) extracted from a nerve tissue a small water-soluble molecule, their "NR" factor, which stimulated the growth of nerve tissues themselves, so that this might be the same as the heterotrophic

factor. On the other hand Hoffman (1950, 1952) and Edds (1953) extracted fat-soluble substances, probably from the myelin sheath, which also promoted the growth and sprouting of axons.

### **22.3. The Vascular Supply and the Control of Local Growth**

Although the vascular system is concerned mainly with systemic controls it does influence growth locally by restricted vasodilations and constrictions. The clearest example is the wave of vasodilation which accompanies the wave of hair growth in rodents (p. 63); this is accompanied by an increased oxygen consumption of the tissues, locally. Growth of the mammalian uterus similarly is accompanied by an increased local blood supply; the growth of the individual foetus in the mouse is related to the blood supply to that particular region of the uterine horn (McLaren and Michie, 1960). Sheep grow more wool in places where they bump external objects (Slen, 1958) and this is attributed to local vasodilation, though here there is also the possibility of stimulation by local wound factors (Needham, 1960a).

Amphibian larvae were found to survive the removal of the heart long enough to show which organs depend most on the blood supply for their growth (Kemp and Quinn, 1954). After fifteen days the growth of the head, eyes and limbs had ceased and the gills had actually regressed in size. Growth retardation was proportional to the magnitude of the normal blood supply to the organ. The localization to particular target organs of the response to certain blood-borne hormones, however, depends on the properties of the organs themselves rather than on their blood supply. Their sensitivity varies and they also may vary in their rate of uptake of the hormone.

It is envisaged that this kind of differential, and perhaps even absolute, specificity of response is shown also towards a set of organ-specific growth inhibitors (Warburton, 1955; Weiss, 1955; Glimos, 1958). These are believed to be produced each by the organ on which it acts, but to operate via the vascular system, back on the organ. When part of an organ or one of a set of organs is removed the production of specific inhibitor is decreased, the concentration in the blood falls and the growth of the organ accelerates by negative feedback. In this way, perhaps, the size of organs is regulated. This mechanism can better explain the usual compensatory hypertrophy of the remainder of a depleted organ than previous theories, which in general had postulated that the loss or damage caused a reduction in output of essential products, or of other services of the organ, and that this in turn caused a demand for them in the body. It was not clear how this induced the necessary compensatory growth but still more seriously those theories could not cover organs such as the testis and mammary gland which pass their products to the exterior. The present theory supposes that all organs pass the specific inhibitor inwards, into the blood stream. It could also cover functional hypertrophy, supposing that the work function of an organ depresses its output of auto-inhibitor, in the manner usual among simultaneous activities, because they compete with each other. It also best

explains the Halstead effect, namely that an engrafted organ is destroyed more rapidly if the host's homologous organ also is present. As an organ grows its total output of auto-inhibitor increases and the termination of growth therefore may be automatic; the factor could control the whole growth programme of the organ.

Positive evidence of such organ-specific inhibitors has been obtained from work on testis, liver, kidney, spleen, and some other organs of mammals. It cannot be considered in detail here, but seems sufficiently adequate to justify the general thesis. In some instances it has been supported by parabiotic, cross-circulation experiments between two individuals: these have shown that the factor is sharply organ-specific and not individual-specific. Perhaps the greatest weakness of the idea as a general phenomenon is that it demands so many factors, one for every organ, at least, and perhaps one for every tissue. Those such as connective and vascular tissues, which occur in every organ would need to be affected indirectly via the chief cell of the organ or to have a different specificity in each organ. The variable fraction of the serum globulin is probably adequate in bulk, and variety of proteins, for the latter to include such a host of factors, but the subject merits further investigation (Goldstein, 1960). From studies on regeneration in Amphibia (Vorontzova and Liosner, 1960) it appears that there is more compatibility morphogenetically between the different tissues of one organ than between the same tissue from two different organs, and this encourages the idea of a single specific factor for each organ, irrespective of its tissue composition. The total number of factors, therefore, may not be incredibly large after all.

It would give Occam's satisfaction if the organ-specific inhibitor proved identical with an already known organ-specific substance. The spleen inhibitor, in fact, is thought to be identical with its organ-specific antigen, which does also inhibit its growth. Both inhibitor and antigen appear at the same stage of ontogenesis and both are taken up again from the circulation, differentially by that one organ (Ebert, 1954a). This specific uptake should allow very low absolute concentrations to be effective, and it eases the quantitative aspect of the problem raised above.

It might be suggested that a locally diffusing substance should be adequate to control growth within an organ, without systemic transport, but in this case the concentration per unit mass of the organ would not be affected by partial organectomy. Its concentration in the blood provides a yardstick of general body size against which to regulate the further growth of the organ. In this event adequate hydration during regeneration (p. 267) acquires an added significance, since it ensures adequate dilution of the inhibitors (Glinos, 1958). There may also be another reason for systemic distribution of the factor, namely the facility it provides for other organs to keep a watching brief over the growth of each one. This might be an important aspect of the control of relative proportions between the various organs, a multilateral system rather than a number of independent unilateral relations between organ and blood.

The simplest kind of multilateral control would be one in which each specific factor inhibited the growth of its own organ but stimulated that of all others. The general serum inhibitor (p. 428) might then provide the necessary preponderance of inhibitory control for the absolute termination of growth. With the discovery of the general growth-promoting action of the salivary glands (p. 376) the idea becomes more plausible for organs in general. The general growth-promoting action of the anterior pituitary growth hormone (p. 365) may have originated from this same property, specially enhanced in this one organ. The enhancement is typical of the evolution of endocrine organs, which originally had another function and specialized their endocrine function as this became obsolete. Here in the pituitary, and perhaps in general, the endocrine function always existed and merely hypertrophied as the main function atrophied. The vertebrate pituitary may have come to relieve most of the other organs of their general growth-promoting function: this would make for economy and simplicity.

However, the role of the pituitary is much more complex than this. In addition to the general action of its APGH factor on the growth of all organs it controls the growth of certain of the other endocrine glands, each by a specific tropic factor; this factor stimulates the activity, and hormone output, of the other endocrine organ and its hormone then acts back on the pituitary to depress the further output of the tropic factor. This is a two-stage negative feedback, in contrast to the one-stage mechanism of the organs in general. Moreover, the pituitary appears to lack an effective auto-inhibitor so that if the ovarian hormone is diverted through the liver and there destroyed, the absence of its normal action of inhibitory feedback on the pituitary permits the latter to develop an adenoma of the cells producing the gonadotropic hormone. If this kind of mechanism has in fact evolved from the simpler general system envisaged above, two profound changes must therefore have occurred. This is possible but not very plausible. There is, of course, a logical weakness in the general theory, in the idea that the growth of each organ is stimulated by all the different factors produced by the other organs, and the correct solution to the general phenomenon may also clarify the special case of the pituitary.

Rather paradoxically the organ-specific inhibitor appears to stimulate growth in the same organ of a young individual (Ebert, 1954*a*; Weiss, 1955). This age difference in response may depend on a difference in sensitivity, since adult tissues are known to be more sensitive to inhibition than those of young animals (p. 89), though this cannot be the full explanation. Returning to the hypothetical expression for growth rate:  $dx/dt = (k/a)(x)(a - x)$  (p. 14), there is the implication that supplementing  $x$  in the early stages should stimulate, and in later stages inhibit, growth. Many substances stimulate in low concentration and inhibit in higher dosage and the higher threshold for inhibition in the young might assist one factor to stimulate then and to inhibit later. It could thus control growth throughout the life cycle, as implied for  $x$  in the expression above and would effect economy in controlling agents. This kind

of change in threshold may explain also the reversal in response, by the body in general, to the thyroid hormone (p. 373). At first it seemed that the salivary gland factor was unique in stimulating growth in the same organ even in the adult (Teir, 1952) but Tasaka found that administered *parotin* actually caused degeneration of the parotid gland (Baker and Pliske, 1957), and partial sialadenectomy caused the remaining salivary glands to proliferate (Alho, 1961). It will be recalled (p. 353) that nerve tissue produces autostimulating factors, which appear to be effective on mature tissue.

It is clear that differential growth is very much under local control, in which all organs and tissues participate in a number of ways. Within an organ one part accelerates the growth of another if for any reason this is younger than itself, and it retards the growth under the converse conditions. The balance between organs may be maintained by the interplay between an auto-inhibitor produced by each organ and hetero-accelerators produced by all the other organs. Systemic controls may be necessary in addition, to make a completely efficient mechanism. The endocrine system may have its own special modification of this mechanism.

In addition, the vascular and nervous systems have locally differential effects, the former by controlling the quantity of oxygen supplied to the tissues, the latter by the secretion of a substance stimulating the growth and maintenance of its end organ. In the C.N.S. one nerve cell affects others in a similar way. The tropic factor concerned is probably not identical with the transmitter substance for nerve impulses, and conceivably may be different at the various sites, like the organ-specific inhibitors. It is probably always a stimulatory factor.

The chemical and physiological relationships between these various local factors promises to be a fruitful field for further research. As already indicated (p. 350) those transported by the vascular system may prove more closely related to the hormones and other systemic factors than to those of local cellular and tissue interactions. These systemic chemicals will now be considered.

## CHAPTER 23

### *The Control of Growth at the Systemic Level*

CONTROL at this level depends to some extent on the nervous system, through its central integration (p. 384), but mainly on blood-borne agents in the vascularized animals. From the previous chapter it is evident that there is no absolute distinction between completely localized, and disseminated effects due to agents distributed in this way, and an action may be arbitrarily defined as systemic if it affects at least two structures not in direct contact. Most of the hormones act systemically and they form the main interest of this chapter. First, however, there are other systemic mechanisms which link up with some of those of the previous chapter.

#### **23.1. General Systemic Controls**

In part, at least, growth is controlled systemically by a simple competition between organs, and activities, for distributed nutrients. The diurnal rhythm in rate of cell division (p. 18) indicates a fairly direct competition between growth and the work functions. Heavy muscular activity suppresses cell division in tissues throughout the body (Bullough, 1952; von Bertalanffy, 1957). In fish, Brown (1946) has demonstrated a critical competition between growth and such work functions as locomotion. Seasonal cycles in the growth rate of children may depend on seasonal changes in the demands of other activities (Fitt, 1941). It is likely, though not specifically shown in every case, that circulating nutrients determine this effect. Probably even the effort of digestion competes with growth since Blumenfeld (1944) found mitosis rate inversely proportional to food intake.

One type of growth may compete with another, for instance regenerative with normal growth and neoplastic growth and with asexual reproduction (Needham, 1960a). The new growth of the foetus in a pregnant mammal inhibits tumour growth in the mother. Productive activities such as lactation suppress her hair growth (von Bertalanffy, 1957).

Other systemic factors depending on the vascular system include the storage and transport of the general inhibitory agent of the serum (p. 428), and the metastatic distribution of tumorous cells, an added reason why cancer is more dangerous in the higher than in the non-vascular animals. It is possible (Wyburn-Mason, 1950) that plethoric individuals are more prone to cancer than those with a less dilated vascular bed. In insects another interesting aspect of vascular control depends on the blood cells or haemocytes (Wigglesworth, 1959). If these cells are "saturated" or "blocked" by ingesting injected Indian ink, or iron

saccharate, growth is completely arrested for several weeks. In *Rhodnius* they must be blocked within four days of taking its usual one meal per instar if the next growth cycle is to be prevented. It may be through this haemocyte mechanism that growth and regeneration compete.

### 23.2. Hormones in the Control of Growth

The action of most hormones is very widespread, that of the APGH (p. 365) affecting virtually every organ and aspect of metabolism in the body. Further, virtually every recognized hormone in the vertebrates affects growth, and collectively they form a highly integrated endocrine system (Table 23.1). It has now been shown (Baker and Pliske, 1957) that the parotid salivary gland produces a systemically distributed hormone which exerts a control over growth as powerful and widespread as that of the typical endocrines and an equal importance may be expected for other organs as yet unrecognized in this capacity.

Hormones are certainly systemic agents in mode of distribution, in field of action and in their interactions, but the actual site of action is the individual cell. Like nerves and their transmitter agents, hormones probably act on the cell surface (Christensen, 1962), triggering off or modifying the complete response of the cell, in the present instance its growth response. Some of them, such as acetylcholine, have molecules small enough to enter the cell and so may act at the subcellular level; there is evidence that acetylcholine will release products of synthesis from the ergastoplasmic membranes (p. 333), though in its normal transmitter action ACh appears to act only on the outer surface of the post-synaptic cell (Katz, 1959). Insulin and other hormones change the activity of hexokinase and if this is a direct action it would presumably be intracellular, but the enzyme probably plays a part also in the transport of materials across the cell membrane. It has even been suggested that hormones themselves act as enzymes, but until recently there was no very convincing evidence for this (Jensen and Tenenbaum, 1946; Sevag, 1951; Barker, 1951). Talalay and Williams-Ashman (1960), Burzatta (1961), Villee (1962) and others find that the steroid hormones affect the pyridine protein dehydrogenases (p. 308) in an enzymic or coenzymic manner and Villee believes that other hormones may behave in a similar manner. Sickevitz (1962) suggests that the hormones in the higher animals functionally replace the enzyme induction mechanisms of microorganisms: of course these are not necessarily themselves enzyme mechanisms.

The reasons for suspecting an enzymic function are that hormones are usually protein or, like thyroxin, are conjugated with protein. There is some reason to think that the steroid hormones are similarly conjugated (Roberts and Szego, 1953), and likewise the plant auxins (Wain, 1953). The association recalls that between co- and apo-enzyme, and it is also significant that a steroid component has been detected in the parathyroid hormone (Rasmussen and Westall, 1957) previously thought to be pure protein. Moreover, the promotion

TABLE

## Summary of the Main Functions of Hormones

To show: (1) resemblances between the two phyla, (2) interrelationships between the actions

Hormone	Action				
	Growth in General	Growth of Organs	Tissue Growth and "Production"	Growth in the Cell	Cell Organelles
Somatotropin (APGH)	+ve (except early)	+ve on bones, cartilages, liver, viscera	+ve on erythropoiesis	+ve on division; +ve on maintenance of gland cells	
Thyroxin	+ve early; later inhibitory (differentiation) +ve on inverters	+ve on nervous system, bones, feathers	+ve on milk and egg production	+ve or -ve on cell division; +ve on cell growth	Attaches to mitochondria, causes swelling, and increased activity
Insulin	+ve (?) conditions	-ve on some bones (micro-melia)		+ve on division	
Parotin	+ve on body-weight	-ve on thyroid, and pituitary ( $\alpha$ -cells)		+ve on growth of nerve fibres, and nerve cell proliferation	
Thymus	? +ve or -ve (+ve with APGH); anti-maturation			? +ve on division	
Pineal	(?) +ve				
Parathyroid	+ve or -ve according to conditions	+ve on bones (normally); +ve on callus formation			
Mineralo-corticoids	+ve on growth and regeneration	+ve on bones	+ve on milk production		? All steroids act at cell and intracellular membranes.
Androgens	+ve, and on regeneration; later inhibitory (fusion of epiphyses)	+ve on target organs, and others, e.g., muscles, bones	+ve on erythropoiesis, skin-, hair-growth	+ve on division	
Oestrogens	? Inhibitory	+ve on target organs	-ve on erythropoiesis	Proliferation in target organs, ? and more generally	? Affect permeability of cell and mitochondrial membranes
Glucocorticoids	Inhibitory, and on regeneration (except early stages)	+ve on bones		Inhibitory on proliferation	
Corpus allatum H.	? Effect on moulting; anti-differentiation			? No effect on cell division	
Prothoracic gland H. (ecdysone)	+ve on moulting, growth and differentiation			+ve on spermatogenesis, and on cell division	
Sinus gland H. (MIH)	-ve on moulting and body weight; -ve on regeneration				
Y-organ H. (MAH)	+ve on moulting and (?) on growth; +ve on regeneration				

23.1

**Affecting Growth in Vertebrata and Arthropoda**

of the various hormones, (3) manifestation of effects at many levels.

on

Vitamins and their Activity	Carbohydrate Metabolism	Lipid Metabolism	Protein Metabolism	Nucleic Acid Metabolism	Metabolism of Other Components
Increases pyridoxin requirement; retains nicotinic acid	Synthesis (inhibits hexokinase)	Catabolism	"Retention"; synthesis; virus growth in host	RNA synthesis; DNA synthesis	Water retention; Ca and P uptake
Increases CoA activity Increases demand for anti-oxidants	Catabolism	Catabolism	Retention	Promotes RNA activity, and synthesis	Water retention; phosphorylation; activity of cytochrome system
? Anti-nicotinic; phosphorylation of thiamine	Economical utilization (hexokinase activation)	Synthesis	Retention; a.a. incorporation		Promotes phosphorylation
					Ca uptake and deposition
				Store of DNA	
					? Controls aldosterone production in adrenal
	Anabolism or catabolism according to concentration		+ve on synthesis		Ca-balance P-balance
Decrease in riboflavin; synergistic with folic acid and E			Retention and synthesis; virus growth in host		Water and salt retention
Synergistic with folic acid			Synthesis in target organs; -ve on collagen synthesis	RNA synthesis in target organs	Na retention
Deplete C (and GSH); redox activity with NADP	Synthesis and storage	? Synthesis	Catabolism; depresses synthesis	Catabolism of RNA; ? no effect on DNA	
	Catabolism, high blood sugar	Storage in fat body and eggs	Synthesis	RNA synthesis	Synthesis of ~P; water retention
				RNA synthesis	Promotes cytochrome system activity
	High blood sugar; +ve on chitin formation	Carotenoid storage; retention of lipids	Retention		Water loss; high blood Ca; phosphorylation
			Synthesis		? Increases blood-P

of oxidation by the thyroid hormone (p. 375) may be due specifically to the iodine radicals of the thyroxin moiety, rather in the manner of prosthetic groups. Nevertheless, even in this case, Barker (1951) had to confess that, in spite of its manifold and fundamental metabolic actions, thyroxin had never been shown to participate directly in any particular chemical reaction. There are many conjugated proteins which are not enzymes.

The action of a hormone might well appear to be enzymic if it did nothing more than control one enzyme, but in fact all seem to have much more comprehensive and manifold actions. This confirms the view that their primary action is at the cell level, or higher, so that there is a cascade of secondary manifestations at lower levels. The multiplicity of effects is increased by the interactions of hormones with each other: more than one affects the activity of hexokinase, the retention of nitrogen, and so on (Table 23.1). Their actions are also too variable to be concerned solely with one or even a few metabolic reactions. A hormone may accelerate growth in one tissue and inhibit in another; the gonadal hormones promote growth in the sex-specific tissues of one sex and inhibit that of tissues characteristic of the other sex (Roberts and Szego, 1953). The thyroid may promote the growth of a particular tissue in the young animal but not in the adult. A change, even to the extent of a complete reversal of response, may occur within the short time of a laboratory experiment; administered adrenal cortical hormones caused depletion of nitrogen and inhibition of the growth of rats, for seven days, but then the response became reversed (Kochakian and Robertson, 1951). Reciprocally, thyroid hormone improved nitrogen retention at first but later the control animals retained more than the experimental (Koger *et al.*, 1942); similar acclimatization was found in the nitrogen-retaining action of the APGH factor, and of the androgens. Some steroid hormones have been found to accelerate a slow process but to inhibit the same process when its rate is high (Dirscherl *et al.*, 1952); this savours of control at a very high level (p. 428). Equally paradoxically, thyroxin in physiological concentration may spare nitrogen but when in either lower, or higher, concentrations may squander it (Carter and Thompson, 1953, p. 214). Ottaway (1953) has shown how some of these paradoxes, at least, may depend on hormones affecting the production or the liberation of each other, in a complex meshwork of systemic feedbacks.

Further evidence that the control as a whole acts at the systemic level is the fact that APGH and the androgens may increase nitrogen retention even without an increase in nitrogen intake (pp. 189, 370), that is without an effect on the reactions of protein synthesis (Gaunt, 1954). Other mammalian hormones similarly act on metabolic turnover as a complete mechanism. This makes the precise mode of action all the more enigmatic, perhaps, but it is conceivable that the economy in nitrogen metabolism is a response of each individual cell; except in the special case of the nervous system, organs do not appear to have any central body which could act as intermediary between systemic agents and their individual cells.

**23.2.1. Hormones Controlling Growth in Vertebrates**

The most important of these is the anterior pituitary growth hormone, APGH, or somatotropin, which promotes the growth of all somatic tissues (Table 23.1, p. 362). The dwarf (Fig. 23.1) resulting from a deficiency, and the



FIG. 23.1. ACHONDROPLASTIC DWARF

The condition is due to deficiency of somatotropin, APGH. This girl is 16 years old and the retardation is limited to somatic growth. Note the relatively large head.

(From Selye (1947), Textbook of Endocrinology (*Acta Endocrinologica, Montreal*), after E. P. McCullagh)

giant (Fig. 23.2) from an excess, of the hormone during the normal growth period in children, as well as the acromegalic (Fig. 23.3) due to excess after this time, are well known because of their characteristic facies. Bone growth particularly is affected, so that the giant has relatively long limbs, especially the distal segments, and the acromegalic shows lateral outgrowth of bones which can no longer grow in length. The effect is most startling in the face, where the smallest of changes are noticeable, but it also affects the other extremities. In

addition, there is visceromegaly, of spleen and liver in particular. Anterior pituitary deficiency in adults causes emaciation, as in Simmond's disease (Fig. 23.4). It may be significant that the dinosaurs had a very large hypophyseal recess, and therefore presumably a large pituitary gland (Thompson, 1942, p. 264). Experimental administration of the hormone to rats produced giants of 700

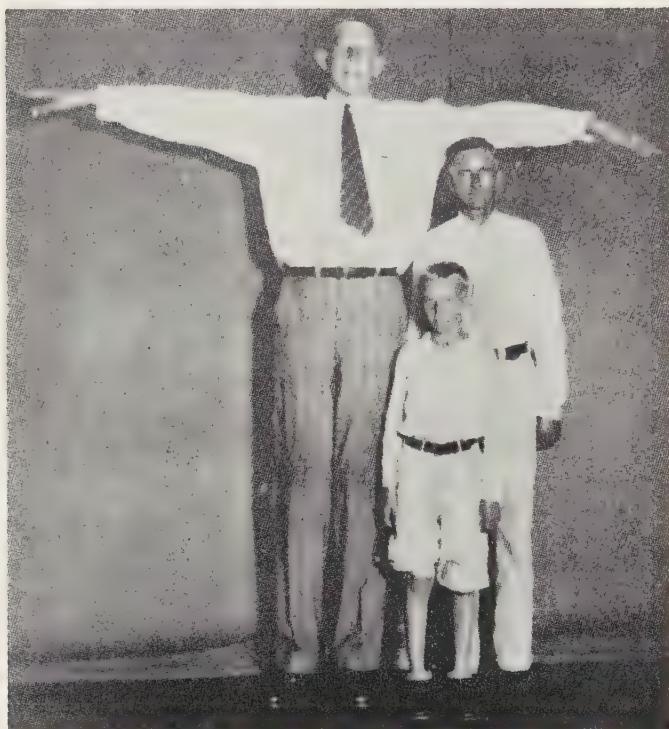


FIG. 23.2. GIANT

The condition is due to excess APGH. The boy, aged 13, is 7 ft 2 inches tall. Note the relatively small head. The other two are his own father and his younger brother.

(From Behrens and Barr (1932), *Endocrinology*, 16, 120)

grammes, compared with the normal 300 grammes of control animals (Beeks *et al.*, 1942) and hypophectomy retards growth in birds (Sturkie; 1954). The hormone has been shown to promote the growth also of fishes and amphibia. It delays amphibian metamorphosis and prolongs the life of animals given supplementary amounts of the hormone (Robertson, 1923; Friedman and Friedman, 1963); it is therefore to some extent a "juvenile" hormone (p. 381). Claims that it stimulates the growth of invertebrates (Hanstrom, 1939) need confirmation; usually the gland was fed to animals, in the early experiments, and can scarcely have stimulated growth except as a non-specific food protein.

Like most hormones, except thyroxin and some steroids, APGH is broken down in the gut.

The extracted hormone does not greatly stimulate growth *in vitro* but a living explant of the gland does (Willmer, 1953, p. 77; Moon and St. Vincent, 1956); presumably the free hormone is rapidly destroyed, or on the other hand may be inhibitory in the concentrations used. It is said to speed cleavage in the egg of *Echinus* (Hanstrom, *l.c.*); this is interesting because there is no growth in the embryo at this stage so that the action must be on nuclear growth or on



FIG. 23.3. ACROMEGALY

Overgrowth of bones in an adult, due to excess APGH. The lower jaw is particularly affected in this condition.

(From Selye (1947), after H. Lisser and M. N. Goldberg)

cell division. The case is also interesting because apart from this there is no good evidence of an effect on early development (Comfort, 1956, p. 187), just as there is no marked effect on early stages of regeneration (Needham, 1960a), on wound healing (Cuthbertson, 1957) or on neoplastic growth (Schulman and Greenberg, 1949). It can cause tumours, however, by chronic administration to rats (Li, 1950). It increases erythropoiesis (Fruhman *et al.*, 1954) and has been shown to increase cell proliferation in the adrenal cortex and other tissues; here the primary effect almost certainly is on cell growth, and not on division. It would be interesting to know if the effect on the adrenal cortex is independent of that of the ACTH factor (p. 370).

APGH appears to increase the yield of virus from vertebrate hosts (Bauer, 1952), which perhaps indicates how fundamentally viruses affect the host's metabolism (p. 156). Virus growth is synthesis at the molecular level and there is abundant evidence that in mammals the hormone induces a net increase in

protein, essentially by reducing the loss of protein from the body; it does this partly by increasing the use of fats as an alternative fuel. It also appears to promote synthesis of protein since it increases the uptake of amino acids from the plasma by the tissues, and promotes the use of glutamine (p. 292) for trans-

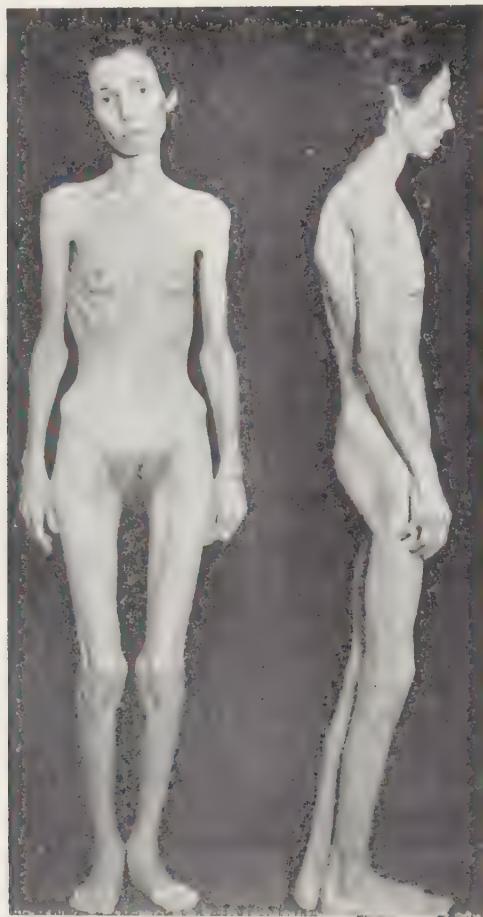


FIG. 23.4. ANOREXIA NERVOSA

A pituitary deficiency disease of adults, closely resembling Simmond's disease, but in which psychological factors may cause the pituitary hypofunction.

(*From Selye (1947), after E. P. McCullagh*)

amination, in the synthesis of amino acids. Its outstanding function in promoting ossification and bone growth depends on an adequate supply of protein (Irving, 1957). There is also an increased rate of uptake and turnover of calcium and phosphorus, increased activity of alkaline phosphatase and of plasma phosphate, and decreased excretion of phosphate. APGH depresses the use of

carbohydrate as fuel and its apparent diabetogenic effect is due to the increase in ratio of fat to carbohydrate used. It promotes water retention by the body, an effect recalling the action of the posterior pituitary antidiuretic factor; it may be independent of this, however, since a number of other hormones and of vitamins also improve water retention. It also reduces the rate of excretion of nicotinic acid (p. 308) and so increases its activity. In addition it is thought to promote the synthesis of both RNA (Geschwind *et al.*, 1950) and DNA (Bergereard and Tuchman-Duplessis, 1953).

Although perhaps not next in importance for growth, insulin, the main pancreatic hormone, interacts so extensively with APGH that it is most conveniently considered here. It has been shown to promote growth (Greco *et al.*, 1942; Prosser *et al.*, 1950, p. 727) though the children of diabetic mothers are sometimes larger than normal (Elrick, 1953), perhaps when the condition is due to an excess of APGH rather than to a deficiency of insulin. An excess of insulin inhibits the growth of the legs of chicks (Landauer, 1953), perhaps indicating a tilt of the APGH/insulin balance in the other direction; this might explain why a deficiency of nicotinic acid also produces this condition of micromelia in chicks (p. 308).

Insulin promotes cell proliferation (Bullock, 1949; Leslie and Davidson, 1951), and at the molecular level it improves nitrogen retention (Jensen and Tenenbaum, 1946; Leslie and Davidson, 1951), peptide synthesis (Krahle, 1953) and the incorporation of amino acids into tissues (Sinx *et al.*, 1952). In these respects it resembles APGH, as also in depressing the amino acid content of the blood; it represses the catabolism of amino acids by deamination (Bach and Holmes, 1937). In contrast to APGH it promotes the use of carbohydrate as a source of energy, and the synthesis of fat from carbohydrate (Gurin and Crandall, 1951). The utilization of carbohydrate under insulin control is very efficient: uptake by the tissues is stimulated and hypoglycaemia is the classical effect of injected hormone. It increases the activity of hexokinase, a key enzyme in mobilizing carbohydrate by phosphorylation, and APGH possibly exerts a directly antagonistic effect here, by depressing the activity of the enzyme. Perhaps indirectly, through this pathway of phosphorus mobilization, insulin promotes the phosphorylations of thiamine, ADP and creatine. The action on thiamine presumably accelerates the conversion of carbohydrate to fat. The effect on growth is thus through general metabolism, with some emphasis on that of energy supply. The beneficial effect on nitrogen anabolism, however, seems as great as that of APGH and it is rather surprising that insulin has not been shown to accelerate general body growth more markedly. Perhaps this is in some way related to the protein-depleting action of glucagon, a second pancreatic hormone (Miller, 1960).

The action of the "S" group of adrenal corticoids, the glucocorticoids, which include cortisone, virtually completes a functional triangle with APGH and insulin, since these corticoids promote protein catabolism (Russell and Wilhelmi, 1941) in favour of synthesis and storage of carbohydrate, and

probably also of fats, though this last is not very clear-cut. The pituitary tropic factor, ACTH, which stimulates the production of corticoids in the adrenal cortex, has virtually the same effects on metabolism, and no doubt operates only through these hormones.

On account of their protein- and NA-squandering action (Pirozynski and von Bertalanffy, 1953) the glucocorticoids tend to inhibit growth in the young (Gaunt, 1954) and to reduce the body weight of adult mammals (Kochakain and Robertson, 1951). They speed the early, regressive stages of regeneration and consequently the process as a whole, though on the later, growth processes their action, as usual, is depressant (Needham, 1960a). Cortisone, the most important of this group of corticoids, depresses cell proliferation (Bullough, 1952), though it is said to accelerate that of embryonic nerve tissue by as much as eight times (Geiger, 1957). Cortisone also depresses collagen synthesis (Gerarde and Jones, 1953) and bone growth (p. 179).

By contrast, the "N" corticoids, or mineralocorticoids, such as desoxy-corticosterone, appear to have a protein-sparing action and to promote growth (Gaunt *et al.*, 1951); they also promote regeneration (Needham, 1960a), and milk production (Cowie and Folley, 1947). In some cases NaCl therapy alone has been found to restore the growth rate of adrenalectomized animals so that the familiar action of this group on mineral excretion may underlie their effect on growth. Wyburn-Mason (1950) concluded that the steroid hormones, in general, have an action very similar to that of the neural tropic factor (p. 352) but it seems clear that there are at least two very distinct groups of corticoids, and that the action of neither of these is very similar to the neural effect.

The androgens resemble the adrenal N corticoids in their general properties and, when tested experimentally, they appear to stimulate the growth not only of organs specific to the male animal, that is seminal vesicles and prostate, the cock's comb and the antlers of the stag, but also body growth more generally (Brody, 1945, p. 164; Gaunt, 1954), and even in the female. The general promotion of muscle growth might be regarded as an effect rather specific to the male, and bone growth (Gardner and Pfeiffer, 1943) must be adequate for the muscles, but there seems no doubt that the growth promotion is more general: in any event the male is generally the larger, that is he grows more than the female. Androgens promote wound healing and regeneration (Needham, 1960a), a sex-specific property which may well have been subjected to natural selection! A stimulatory effect on integumentary structures (Hamilton and Montagna, 1950) and on red cell proliferation (Taber *et al.*, 1943) also has been recorded. Like APGH, the androgens promote nitrogen retention (Dorfmann, 1949; Rupp and Paschkis, 1954), increase the yield of virus from mammalian hosts, and increase the retention of water and salts by the body. The salt retention recalls the action also of the mineralocorticoids. In the later stages of growth, androgens are inhibitory since they promote the fusion of the epiphysis with the shaft of the long bones (van Wagenen, 1950); in this they resemble thyroxin.

The oestrogens promote the growth of the female-specific target organs, but in general resemble the S corticoids in action. They repress bone growth, at least in those groups which have the male normally larger than the female, and spaying leads to an increase in weight of many organs in the rat (Brody, 1945, p. 164). An inhibitory action on hair growth is found, but presumably excepting that of the "crowning glory." Other inhibitory actions recorded are on regeneration, the growth of embryos and of neoplasms, on collagen synthesis and on erythropoiesis (Taber *et al.*, 1943). The action on the embryo is interesting since there is considerable oestrogen in placental tissue. There are records of growth promotion, however, which do not seem to be restricted to female-specific tissues (Gardner and Pfeiffer, 1943; Bullough, 1952); cell proliferation (Bullough, *i.c.*) and nitrogen retention (Dorfmann, 1949) are proximate manifestations of this. Oestrogens in particular, and also some of the other steroid hormones, promote transhydrogenation between NAD and NADP (p. 308). This is an important metabolic reaction since NADP mainly promotes anabolic and NAD catabolic, reactions (Villee, 1962).

There is not much certain knowledge about progesterone, though it is known to promote the growth of organs specific to pregnancy, and that of the foetus (p. 400). It also promotes the formation of the egg shell in birds (Frazer, 1959). The sequent effects of oestrogens, progesterone and the lactogenic hormone of the pituitary, on duct growth, acinar cell growth and active milk production, respectively, by the mammary glands constitute a very striking example of the synergism between endocrines in the control of growth. In fact, virtually every hormone in the body affects mammary activity in some way (Gorbman and Bern, 1962); no doubt this is not peculiar to this one organ. For details of the sex-specific actions of the gonadal hormones the works of Burrows (1949) and Marshall (1952) may be consulted. The gonadotropic hormones of the pituitary act indirectly, through the gonadal hormones, and produce the same effects.

Probably all of the anterior pituitary factors have some effect on growth, directly or indirectly. The lactogenic hormone, prolactin, in addition to its control of milk secretion in mammals and of pigeon milk, the analogous material from the crop of pigeons, promotes general growth in mice and rats (Bates *et al.*, 1942; Schooley *et al.*, 1941). The control of mammary and crop secretions by the same hormone begs the whole question of the distinction between analogy and homology. Here the target organs are analogous but the hormone is homologous.

Structurally the molecule of prolactin is closely related to that of ACTH (Das Gupta and Young, 1958) and there are similar relationships between other pituitary factors, so that a number may be formed from the same prohormone. This increases the difficulty of deciding how many separate pituitary factors there really are. There are only five or six histologically distinct types of cell in the gland (Barrington, 1963) and these are not sharply segregated from each other. It now seems certain (F. G. Young, 1950) that APGH and the

diabetogenic principle are the same hormone, and further simplifications of the picture may be anticipated. Nevertheless, it is evident, from the variety of



FIG. 23.5. LORAIN-LEVY OR PETER PAN TYPE OF DWARF

The condition is due to deficiency of hormones of anterior pituitary gland in childhood. Both boys are 15 years old. The dwarf has the build of a child of 6 years, and an equally youthful expression. His sexual development is proportionately retarded (cf. Fig. 23.1).

(From Selye (1947), after A. Pinto Viégas)

clinical pituitary conditions, that quite a number of factors are involved. Most of those not specifically considered are crinotropic factors controlling the growth and productive activity of other endocrines, and they may affect growth extensively in this indirect way. The common pituitary dwarf shows

no comparable repression of sexual development whereas the Lorain-Levy type (Fig. 23.5) is a complete Peter Pan. The progeric type (Fig. 23.6) is a prematurely ageing dwarf; it is not clear that somatotropin is mainly at fault here, for there is atrophy of the whole anterior lobe. The posterior lobe



FIG. 23.6. PROGERIA

A condition of severe deficiency of the anterior pituitary hormones in a young girl of 8 years old. In addition to dwarfing there is premature ageing.

(From Starling's Principles of Human Physiology, 12th ed. London, Churchill)

factors seem more concerned with purely physiological work functions, though by their control of water balance and of vaso-motor tone they may well affect growth.

The growth and output of the thyroid gland is largely controlled by the thyrotropic hormone of the pituitary. Thyroxin is one of the major hormones concerned in vertebrate development. Its most interesting property is in

stimulating growth in the young animal and the productive activities of the adult, but also metamorphosis in amphibians (Gudernatsch, 1934; Allen, 1938) and fish, involving differentiation and other exergonic processes. Growth acceleration by extra thyroxin in young mice, dogs, lambs and chickens, and in tadpoles, is familiar knowledge. A similar action in teleost fishes (Hoar, 1957) and other vertebrates also has been shown. There now seems to be good evidence (Schneider, 1940; Srinivasan *et al.*, 1955) that it promotes the growth also of invertebrates (Hanstrom, 1939); perhaps the simple non-protein nature of thyroxin itself accounts for this difference from most vertebrate hormones. The effective concentration for insects is very low, and illustrates the paradoxical properties of the hormone, according to concentration; it possibly also explains why no stimulation was found in some earlier experiments. The productive activities improved by the hormone include milk and egg production by farm animals (Brody, 1945). The hormone also continues to promote regenerative growth in adults (Needham, 1960a).

It promotes the metamorphosis of tunicates (Grave, 1936), and of eels and flatfish (Prosser *et al.*, 1950, p. 729), as well as of the Amphibia. The silverying of salmonid fishes at the time of the seaward migration (Robertson, 1948; Hoar, 1957) is another metamorphic kind of change controlled by the thyroid. The moulting of lizards, which it also induces (Ratzersdorfer *et al.*, 1949) may be regarded as a minor change of the same general kind.

The thyroid hormone affects the various organs differentially. In man, growth of the brain is particularly dependent on this hormone and thyroid-deficient children tend to be cretinous (Fig. 23.7), that is mentally deficient, as well as dwarfed; pituitary dwarfs are much less affected mentally. Marked actions on the growth of bones and feathers also have been found. The action on bone depends on pituitary synergism: alone thyroid has no effect, but together with APGH the growth rate is higher than under APGH alone (Beeks *et al.*, 1942). Like the androgens it also promotes the eventual fusion of shaft and epiphysis (Fig. 4.17), so terminating growth. This may be regarded as a metamorphic change, perhaps, since tadpoles grow indefinitely under the influence of APGH in the absence of the thyroid.

Some have found a positive effect on cell division by thyroxin (Gardner, 1942; Weiss and Rosetti, 1951), but others an inhibitory action (Torrey, 1928; Peter, 1945; Mittler and Herman, 1950.) At the intracellular level it has been found to stimulate the activity of RNA (Pirozynski and von Bertalanffy, 1953). It also improves nitrogen retention, thyroidectomy leading to increased protein catabolism (McElroy and Glass, 1952, p. 894); cretins therefore have low nitrogen retention. Thyroxin promotes transamination and so appears to synergize with APGH extensively at this level also. However, during muscle contraction, and in the early stages of regeneration when APGH is ineffective, it stimulates nitrogen flow from the body (Gribble and Peters, 1951). Like APGH, it promotes fat catabolism, increasing the activity of coenzyme A (p. 313), and it increases water retention.

The main difference from APGH at this level is that it stimulates the breakdown of glycogen, also (Kochakain, 1952). It may therefore be included in the list of hormones which have decisive actions on all three main classes of food material, but give a total syndrome which is unique. Thyroxin also increases the activity of phosphatases, the phosphorylation of ADP, and the activities of



FIG. 23.7. CRETINISM

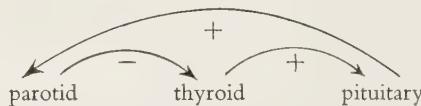
A condition due to deficiency of the thyroid hormone in children. The broad nose, protruding tongue and impression of mental deficiency are characteristic. Retardation of growth in height is not very evident at this age, but the typical protruding abdomen is already seen.

(From Selye (1947), after J.-I. Lobo)

the cytochromes and other terminal oxidases (Tissières, 1946, 1948; Evert, 1953). In fact, the coupling between terminal oxidation and phosphorylation is probably its essential metabolic concern, both in growth and also more generally. Its notorious analogue, DNP, uncouples the two processes, and inhibits growth. In excess, thyroxin itself is an uncoupling agent and this probably explains why only a low concentration stimulates insect growth. It may also help to explain the change in action during the life cycle of a vertebrate, from being

strongly promotor to inhibiting growth; the amount of material in the follicles of the gland increases with age, and it presumably reaches a concentration which uncouples oxidation from certain phosphorylation reactions essential for growth but not from those concerned with other functions. The change in action on bone growth may be part of this same general change, but it is believed (Prosser *et al.*, 1950) that the action on amphibian metamorphosis is quite distinct from that on metabolism. There is perhaps some support for this also in the fact that the hormone does not stimulate metabolic rate in fishes (Barrington, 1952), as it does in mammals; the implication is that effects on metabolism may be more variable than those on growth. The hormone in fact probably depresses metabolism in fishes, at high temperatures, though it appears to stimulate that of invertebrates (Hanström, 1939). The teleost response may be a biologically adaptive one for an aquatic poikilotherm accustomed to relatively low temperatures. Here again, it may conceivably depend on uncoupling, this time of metabolic phosphorylations. It is another of the paradoxes of thyroxin action. A further paradox is that it increases the demand for a number of the antioxidant vitamins; on the face of it this is a useless creation of an unnecessary demand, but one possibility is that the antioxidants control the low redox level of the proximate stages of respiratory pathways, of which thyroxin controls the terminal stages.

The parotid salivary gland may conveniently be considered here since its action on growth involves the thyroid and anterior pituitary in particular (Baker and Pliske, 1957). It has even been suggested that APGH exerts its effect mainly through the parotid. Chronic administration of parotin causes not only degeneration of the homologous organ but also of the thyroid and of the  $\alpha$ -cells of the pituitary, while removal of the thyroid, and still more of the anterior pituitary causes atrophy of the parotid. This has the appearance of the usual indirect, negative-feedback type of control of the endocrines, but possibly a three-stage mechanism operates in this case. If so, then the pituitary would seem to be the gland acting immediately on the parotid, since its removal has a greater effect on the latter than removing the thyroid. The action of the thyroid on the pituitary then must be stimulatory, and so must be due to a factor distinct from thyroxin, which is inhibitory (p. 358). The complete scheme would therefore be —



Alternatively there may be two separate two-stage mechanisms, operating between the parotid and each of the others. The adrenal gland appears not to influence the parotid but the gonadal hormones probably do.

Extracts of the parotid have been found to increase the body weight of rats, and sialadenectomy causes loss in weight. To a considerable extent carbohydrate food can prevent the loss and this, no doubt, is relevant to the fact that an

amylase is the major enzyme of saliva. Parotin improves the uptake of  $\text{Ca}^{+}$  from the gut, and its deposition in the bones. Extracts of the salivary glands are more potent than those of any other tissue in promoting the growth of nerve fibres and the proliferation of cells in the peripheral ganglia (Cohen and Levi Montalcini, 1958), though this agent has not been identified with the sialhormone.

The parathyroid gland is primarily concerned with  $\text{Ca}^{+}$  metabolism, excess of parathormone mobilizing calcium from the bones, with dramatic results, sometimes afflicting man. It inhibits growth (Thompson and Huxley, 1934) probably for this reason. However, under appropriate conditions it will promote the deposition of calcium, for instance in the later stages of normal growth in mammals and following a period of experimental decalcification. This last appears to be the familiar self-reversing or accommodating type of action (p. 364). In any case it is understandable that parathormone is found also to accelerate growth under appropriate conditions, and to improve callus formation in the healing of fractures. It promotes the fusion of shaft and epiphysis, and in this way, again, is growth-inhibitory (Greep, 1949).

A growth-promoting action by the pineal has been detected in fishes (Hoar, 1957), and in some other animals, though there are many records showing no effect, or inhibitory effects, experimentally (Gladstone and Wakeley, 1940). Epiphyseotomy appears to accelerate sexual maturity (Moszkowska, 1955) and this will terminate growth; if the active gland retards maturity it will tend to prolong growth. It probably acts via the pituitary, but also appears to control the output of the mineralocorticoid hormones of the adrenal (Farrell, 1960).

The thymus, supposing that its action is hormonal, which is still uncertain (Anderson, 1932), also delays maturity. It is large in young growing animals, and often atrophies at maturity in those vertebrates with determinate growth. In some, however, it regenerates each year after the breeding season. Like thyroxin it has been found to increase the effect of APGH on growth (Comisa, 1958). No clear effect has been found in isolation (Segaloff and Nelson, 1940) and earlier experiments claiming such an effect may have shown only the non-specific value of the gland as food. By the mass of lymphocytes it produces it may provide a reserve of nucleoprotein to assist in cell division (A. F. W. Hughes, 1952a).

The spleen, another lymphopoietic organ, has been found to promote growth in the salamander and the formation of callus tissue in bone (Kostic and Vlatkovic, 1936).

### **23.2.2. Hormones Controlling Growth in Invertebrates**

In planarians, diffusing factors control regenerative growth (Bröndsted, 1955; Lender, 1952) and a gonadal hormone promotes the regeneration specifically of the penis. The proboscis of the female *Bouellia* secretes a steroid growth inhibitor (Carlisle, 1957) which acts on young larvae settling on the organ; these are caused to mature as males, and it seems likely that this is the primary

effect. Annelids possess a number of groups of neurosecretory cells, mainly in the brain, some of which affect regenerative and other types of growth (Harms, 1947-9; Hubl, 1953; Clark and Clark, 1959; Clark and Evans, 1961). The hormones from these cells travel the length of the body, either in blood cells or possibly along nerve pathways. The arthropods have exploited the neural type of endocrine organs much further, but the cephalopod molluscs appear to

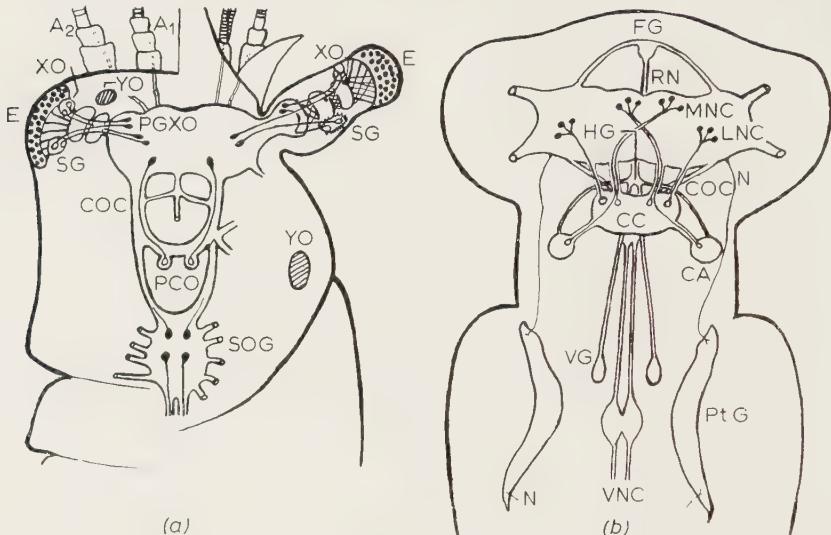


FIG. 23.8. DIAGRAMS OF THE NEURO-ENDOCRINE SYSTEMS CONCERNED WITH GROWTH IN (a) CRUSTACEA AND (b) INSECTA

In (a) the sessile-eyed condition (Isopoda) is shown on the left and the stalk-eyed condition (Decapoda) on the right. A<sub>1</sub> and A<sub>2</sub>, first and second antennae; CA, corpus allatum; CC, corpus cardiacum; COC, circum-oesophageal nerve commissures; E, eye; FG, frontal ganglion; HG, hypocerebral ganglion; LNC, lateral neurosecretory cells of the brain; MNC, medial neurosecretory cells; N, nerves; PCO, post-commisural neurosecretory organ; PtG, prothoracic gland; PGXO, pars ganglionaris of the X-organ; RN, recurrent nerve; SG, sinus gland; SOG, suboesophageal ganglion; VG, ventricular ganglion; VNC, ventral nerve cord; YO, Y-organ.

(Based on various authors)

resemble the vertebrates in developing other tissues, as well as nerve cells, for the purpose. Of the endocrine organs of this molluscan group, the branchial gland appears to produce a growth hormone; moreover if one is extirpated the partner gland shows compensatory hypertrophy. Earlier claims that a gonadal hormone induced regenerative growth in the hectocotylized arm of the male have not been confirmed (Callan, 1939). Little was known of any action on growth by the endocrines of other molluscs until recently (Herlant-Meewis, 1962). Those of the slug, *Arion*, show similarities to the system in insects.

By contrast there is now extensive knowledge of the endocrinology of the Crustacea and Insecta, among arthropods (Hanström, 1939; Wigglesworth, 1954; Naples symposium, 1954; Carlisle and Knowles, 1959; Novak, 1959; Scheer, 1960). As in the vertebrates, most of the endocrine organs (Fig. 23.8) have some effect on growth (Table 23.1, p. 362). In the decapod Crustacea a clear-cut antagonism is found between a moult-accelerating factor (MAH) and a moult-inhibiting factor (MIH), which affect the whole growth complex associated with moulting. Both are produced rather widely in cells of the C.N.S. but especially in groups of cells in the brain. Secretion flows down the axons of these cells to be stored, the MAH in the distal part of the *X-organ* (Hanström's organ) and the MIH in the *sinus gland*. Both storage organs are located within the eyestalk of the decapods (Carlisle, 1953b). The MIH was detected as early as 1905 when Zeleny removed the eyestalks of decapods and noticed the more frequent moulting. An effect of the factor on growth and body weight was shown by Scudamore (1947) and others (Bauchau, 1949). The MAH (Carlisle, 1953a) is less well characterized as yet but it probably accelerates growth proper as well as moulting. It has some resemblance to vertebrate APGH and so has been called somatotropin (Knowles and Carlisle, 1956). In fact, APGH is possibly more potent in moult acceleration than the indigenous hormone (Carlisle and Dohrn, 1953).

The MIH is found to inhibit regenerative growth also (Cornubert, 1952; Bliss, 1954) but it seems to conserve, rather than squander, nitrogen. In general, results have been those of removing the whole eyestalk so that MAH also may have been involved. Extirpation of the stalk causes a decrease in protein content of the body (Nieland and Scheer, 1953) and an increase in nitrogen excretion (Needham, 1955). The eyestalk factors also maintain high levels of sugar and of calcium in the blood (Kyer, 1942), and a low water content in the tissues; this last effect is presumably due to MIH specifically. Virtually all aspects of metabolism are affected by these hormones and there is considerable resemblance to vertebrate systems (Table 23.1, p. 362).

The functional excretory organs of the Crustacea develop in either the antennal or the maxillary segment, and the embryonic pair in the other segment persist (Fig. 23.9) as an endocrine organ (Needham, 1942a), the *Y-organ* of Gabe (1954) or ventral gland (Scheer, 1960). This produces a moult-promoting factor (Knowles and Carlisle, 1956) and it is possible that MAH in fact acts via this organ (Passano, 1960), which produces the definitive moulting hormone. It also appears that MIH inhibits Y-organ activity when sufficiently concentrated in the blood. Light promotes the release of MIH into the blood and this is, no doubt, a reason why the sinus gland is located in the eyestalk. In the dark, therefore, moulting is accelerated (Bliss, 1954), though the hormone continues to accumulate in the gland and eventually it must overflow into the blood; the moulting rate in the dark then falls again. The anecdisis of the winter period appears to be associated with high MIH activity so that in this case a factor other than light must be involved, as also in inducing the shorter periods

of diecdysis between moults during the summer. There may be still other factors concerned with moulting: Scheer and Scheer (1954) visualize the possibility of as many as six. Gonadal and other hormones controlling the

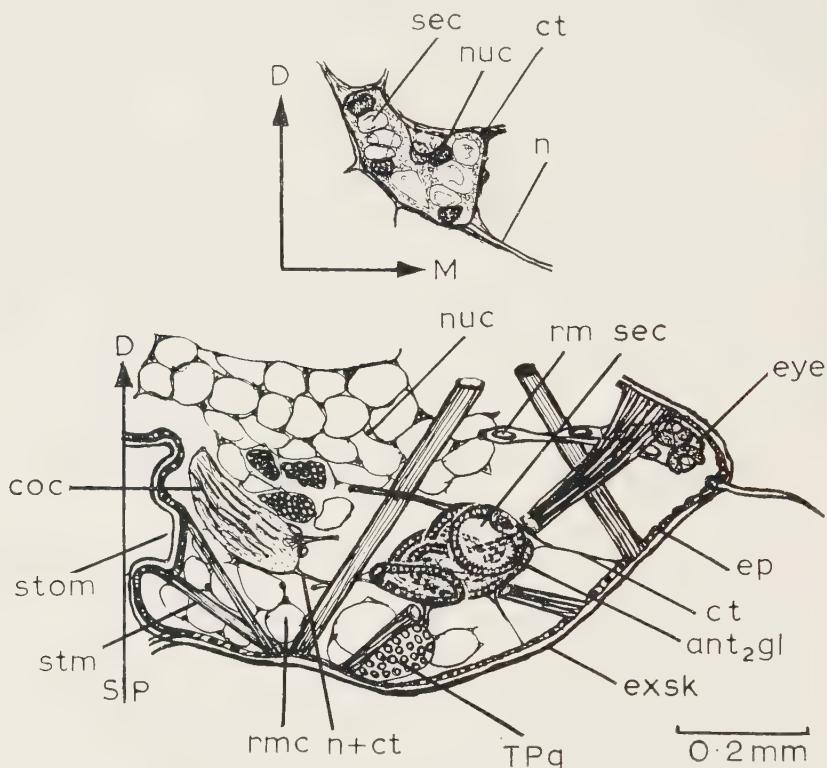


FIG. 23.9. VENTRAL PART OF A THICK TRANSVERSE SECTION THROUGH HALF OF THE HEAD OF THE ISOPOD CRUSTACEAN, *Asellus*, TO SHOW THE LOCATION OF GABE'S Y-ORGAN (ALSO KNOWN AS NEMEC'S ORGAN), IN THE SECOND ANTENNAL SEGMENT (*ant<sub>2</sub>gl*). (Above) Y-ORGAN OF OTHER SIDE OF BODY, IN DIFFERENT PHASE OF ACTIVITY

COC, circum-oesophageal nerve commissure; *ct*, connective tissue; *D*, dorsal direction; *ep*, epidermis; *exsk*, exoskeleton; *M*, medial direction; *n*, nerve; *nuc*, nuclei; *sec*, secretion in vacuoles of cells of Y-organ; *SP*, sagittal plane; *stom*, stomodaeum; *TPg* Ter Poghossian's organ.

(From Needham, 1942a, Quart. J. micr. Sci. 83, 205, by courtesy of The Company of Biologists)

growth specifically of the reproductive organs are now recognized (Charniaux-Cotton, 1956; Carlisle, 1957).

The endocrine system of insects resembles that of the Crustacea in the specialization of storage organs round the ends of the axons of neurosecretory cells, themselves located mainly in the brain. The resemblance naturally is closer to the system in the sessile-eyed Crustacea (Wigglesworth, 1954b;

Bodenstein, 1957; Novak, 1959). A group of neurosecretory cells near the mid-line, in the posterodorsal, or *intercerebral*, region of the brain pass their secretion to the *corpus allatum*, a median unpaired gland, while neighbouring cells pass theirs to the *corpus cardiacum* situated on each side of the anterior aorta. This last hormone, the prothoracotropic factor, is released into the blood and so acts on the definitive endocrine organ, the *prothoracic gland*. The hormone from this is the most important of those controlling growth in insects; it accelerates moulting, growth and differentiation and so is known as the moulting hormone or the growth and differentiation hormone (GDH). The gland is therefore probably analogous to, if not homologous with, the Y-organ of the Crustacea, the *corpus cardiacum* corresponding to the distal part of the X-organ. The *corpus allatum* then might be expected to correspond to the sinus gland of the Crustacea, though it appears to inhibit differentiation rather than growth. In synergism with GDH it causes persistent growth in the existing state of development and so it is known as the juvenile hormone. GDH alone accelerates differentiation, and metamorphosis. It therefore also resembles the vertebrate thyroid hormone, while the allatal factor has some resemblance to the pituitary APGH factor (Wigglesworth, 1954*b*).

GDH resembles thyroxin further in promoting the activity of the cytochrome system, most clearly seen in the breaking of diapause in some insects. Also like thyroxin, it has some feedback control over its own tropic factor. However, it tends to atrophy at metamorphosis (Bodenstein, 1957) and its active principle, ecdysone,  $C_{18}H_{30}O_4$ , is a hydrocarbon derivative, probably a sterol. It promotes spermatogenesis and so has some resemblance to the vertebrate steroid hormones. In those insects which liberate the hormone once in each instar this is followed by a single pulse of cell proliferation in the epidermis and other tissues (p. 21). It promotes the synthesis of RNA and no doubt of protein also. Local wounding causes an increase in activity of the cytochrome system in pupae, possibly through locally produced ecdysone (Shappirio, 1960).

The allatal hormone also has effects which are manifest at various levels (Table 23.1, p. 362), though no clear-cut effect on cell proliferation has been shown. It promotes the synthesis and maintenance of body proteins (Novak, 1959), and the synthesis of RNA and of energy-rich phosphorus compounds. It maintains water content and promotes fat mobilization; carbohydrate breakdown is the main source of energy and the whole syndrome is more similar to that of insulin, perhaps, than of any other vertebrate hormone. However, it causes a high level of sugar in the blood, and has other complicating actions. In the adult, when the juvenile function is redundant, it assumes a promotor action on the development of the accessory reproductive organs and on egg ripening (Novak, 1951). This is more reminiscent of the pituitary, and, like APGH (p. 365), the allatal hormone increases the activity of alkaline phosphatase. The crustacean sinus gland similarly shows a greater variety of metabolic effects, which collectively must almost necessarily show most resemblance to those of

the vertebrate pituitary. The following tentative comparisons therefore may be made—

Vertebrates	Crustacea	Insecta
Pituitary (APGH)	Sinus gland (MIH)	Corpus allatum
Thyrotropic hormone	X-organ, MAH factor	Corpus cardiacum factor
Thyroid	Y-organ	Prothoracic gland

It is clear that there are discrepancies in details: in addition to those mentioned, the allatal hormone stimulates oxygen consumption, a thyroxin-like property. The endocrine systems have evolved independently in the three groups of animal, and while collectively the endocrines of each control the whole of a pattern of metabolism (Table 23.1, p. 362) which is very similar in all, the allocation of control to particular organs has been somewhat different. The true extent of the differences between Crustacea and insects is still a matter for debate but it seems certain that much of their endocrinological evolution has been more recent than the phylogenetic dichotomy between the two.

In some species of insect the female determines whether or no the progeny of her eggs shall undergo diapause (p. 426) during development (Lees, 1955). The determination depends in turn on the environmental conditions of the mother, acting via the C.N.S. and the secretion of a humoral factor by cells of her brain. This passes in the usual way along their axons to be stored in a *suboesophageal gland*, whence it passes to the eggs via the blood. It is active only in the adult female.

Other factors which have been regarded as hormones affect pigment synthesis in the eye of insects. Like hormones they are blood-borne but one, the V<sup>+</sup> "hormone," appears to be kynurenin, an active intermediary substrate in the pathway of synthesis. The pathway varies in details in the different insects, giving a range of eye colours, genetically determined. This appears to be a case of synthesis of organ-specific substances elsewhere, followed by transport to the organ, and not an endocrinological phenomenon; at the same time it is an interesting example of the systemic control of growth.

Enough is known of the hormones of the arthropods to indicate that in the higher invertebrates, as in the vertebrates, each affects directly or indirectly most aspects of metabolism. For further details Turner (1955), Barrington (1963) and other general endocrinological texts may be consulted.

### 23.2.3. Conclusion

A complete theory of the mode of action of hormones is scarcely possible yet, though at least it seems clear that their control acts essentially above the level of the catalysis of particular reactions (p. 364) notwithstanding recent evidence on the steroids (p. 361). The processes affected directly or indirectly by thyroxin,

insulin or APGH collectively employ at least tens of enzymes, and when the amount of a hormone changes, whole pathways of biosynthesis, and not simply particular reactions, are speeded, arrested or even effectively reversed. There is little doubt that the control is imposed at a level higher than that by the transferases (p. 303), for instance.

It is also clear that the endocrine system acts as a fairly well-integrated unit and that this is the main reason why many hormones appear to affect the whole metabolic network (Table 23.1, p. 362). In many cases (p. 364) the hormones control each other through their rate of production (Ottaway, 1953), rather than by direct antagonism or synergism in their subsequent functioning. In other instances more direct interactions seem the only reasonable explanation of the facts, for instance the antagonistic effects of APGH and insulin on hexokinase activity. However, the action on hexose metabolism is such a small part of the total action syndrome of the two hormones that it is difficult to believe that the antagonism is as simple and direct as it seems.

The suggestion (p. 361) that hormones might act mainly or even solely at the cell surface has the virtue of supporting the intuitive conviction that living organisms must be able to control their growth at key points above the level of individual reactions. There is considerable evidence in favour of the suggestion (Rosenberg and Wilbrandt, 1952; Christensen, 1962), in many cases. The insulin molecule is probably too large to pass through cell membranes though some, such as the posterior pituitary factors and the steroids, are small enough; this presupposes that the steroids are not rigidly bound to a large carrier molecule. Insulin has been found to promote the uptake of glucose by the cell and this could be a direct result of an action on the cell membrane. Thyroxin promotes the uptake of food from the gut (Brody, 1945, p. 172), and the artificial oestrogen, stilboestrol, accelerates the uptake of phosphatide by cells, from the blood (Hevesy, 1948). Steroids are surface-active agents and are probably normal constituents of cell membranes (Booij and de Jong, 1956) so that they might well control transport across the latter. The neurohumors, acetylcholine and adrenalin act primarily at the outer surface of the cell membrane (p. 361), notwithstanding their small molecular size. As intercellular messengers in their evolutionary origin, it seems inevitable that their first action must be at the cell surface, though this is not necessarily their only site of action. In cells with an effectively extracellular labyrinth (Fig. 11.2) hormones could have access to most intracellular activities without ever passing through any membrane; acetylcholine might in this way exert its normal membrane action on the ergastoplasmic system (p. 334). The accumulation of thyroxin on the mitochondria (p. 375), however, perhaps implies penetration into the cytoplasm proper.

Action at the cell surface might explain the manifold effects of each hormone, since all could thus control much of the metabolism of the cell, but it only emphasizes the difficulty of explaining the differences in action between the various hormonal "principles." Selective action on particular patches of the

membrane is a possibility already considered in the general theory of membranes (Davson and Danielli, 1952). There may be permeases (p. 185) specifically sensitive to particular hormone actions, determining what substrate materials enter and what products leave the cell, as well as enzymes like hexokinase, concerned with permeation in general.

It is now clear that the hormones of insects (Schneiderman and Gilbert, 1964), and no doubt of other groups, activate particular chromosomal loci, so that they may enter the cell and even the nucleus. However, it is still possible that the effect is indirect, through changes in cell permeability to more proximate agents, in some cases possibly as simple as Na and K. The loci activated vary with the target organ, so that the action is not as invariable as a direct effect of the hormone should be.

It is evident that much of the control of growth at the higher levels depends on the two main co-ordinating systems of the body, the nervous and endocrine systems, just as in other activities, and there is much the same division of labour, the nervous system controlling localized, specific responses and the endocrines more widespread activity. In growth the endocrine system is possibly the more important whereas in the typical effector functions, such as locomotion, the more sharply localized responses are the most important, as well as the most highly evolved. Even considering growth control as a purely internal matter it is clear that the endocrine system plays a leading part, and this becomes perhaps even more certain when the action of external agents (p. 398) is added to the picture. However, the endocrine system rarely has its own afferent components and uses mainly the afferent nervous system in its responses to external agents. The afferent nervous system therefore is one of the systems controlling growth at the systemic level, in its orthodox capacity of impulse conduction; at the same time it is also a system for the local control of growth, through an antidromic action on its sensory end organs (p. 353).

## CHAPTER 24

### *The Genetic Control of Growth*

IN the genome is the codification of the entire intrinsic mechanism of control, determining the whole pattern of growth, up to the organismal level, except in so far as the pattern is modified by external factors (p. 398). In microorganisms, the organismal is also the cellular level and the code contains only genes acting at this or at lower levels. Indeed most of the genetic factors which have been studied in microorganisms act directly on individual chemical reactions and there is considerable justification for the kind of aphorism "one gene—one reaction," even to the extent of using it reflexly as the definition of the gene. In the Metazoa, however, hormonal, nervous and other intermediary controls already considered have been superimposed on those at the cellular and molecular levels, and the mechanism of this superimposition is the second if not the major problem of this chapter.

It is known that controls acting at these higher levels in Metazoa have come to be determined by discrete genetic mechanisms, in many cases mainly by a single Mendelian factor. Mutant phenotypes of a number of these are due to a change in the action of a particular endocrine (Stockard, 1934; Dickerson, 1954; Haldane, 1954, p. 29); thus dogs of small breeds have large thyroids and a particular dwarf mutant in mice can be made to grow normally by the administration of additional APGH. Again, selection for rapid growth rate in pigs was found to have involved selection for high APGH output (Dickerson, l.c.). The "lethal giant" mutant of *Drosophila* also may be hormonally mediated (J. Needham, 1942, p. 362), presumably depending on an excess of juvenile hormone relative to GDH (p. 381). It is clear, therefore, that the Metazoa have evolved some genes which act primarily at the highest, systemic level of control, and it will be seen that they have also genes specific to each of the other, intermediate levels.

There are other aspects of the phenomenon of superimposition in the Metazoa: in microorganisms the life cycle is little more than a bout of biosynthetic activity but in the metazoan there is superimposed a relatively long ontogenetic process, with progressive deputization of control from a main organizer to those of successively lower grade. In addition to its time-sequence this also has its spatial aspect, since the main and subsidiary organizers are localized in space, and form-change, or morphogenesis, is a major aspect of development. This spatiotemporal hierarchy of organizer action must be distinguished from the hierarchy of levels of growth-control recognized in the preceding

chapters. Consequently there may be time (or stage)-specific, site-specific, and level-specific genes in the Metazoa, in addition to the reaction-specific genes of microorganisms.

The genes controlling growth show the orthodox genetic properties, which is only to be expected since most genes control developmental processes, in a broad sense. Many growth mutants, for instance dwarfism in maize, are controlled by a single Mendelian gene pair (Wardlaw, 1952, p. 139). In other cases, however, (Dickerson, 1954), including that of melanin synthesis in mammals (Wright, 1941; Fitzpatrick *et al.*, 1958), a large number of genes are involved; reciprocally one growth gene may have pleiotropic effects, on a variety of processes (Goldschmidt, 1938, p. 114). The hybrid between the Flemish Giant and the Polish breed of rabbit grows at a rate intermediate between that of the two parents, while the short, bent character of the legs of the bassett hound is almost completely dominant to the long-legged condition of the saluki. Platfish-by-swordtail hybrids grow at the rate of the latter species but certain particular organs at the rate of the platyfish (Gordon, 1957). It is possible to select for growth genes, but in such domestic animals as the pig, selection for rapid growth has already reached its limit, modern breeds being presumably homozygous for all relevant genes.

There are some properties of growth genes, however, which may be peculiar to them or are more easily recognized than in other types of gene, because the phenotypic expression can be measured so quantitatively. They have special interest to the geneticist, therefore, as well as to the auxologist. One is hybrid vigour, or heterosis, the very common tendency for a hybrid to grow faster, reach a larger final size, show greater productivity and live longer than either of its pure-line parents. It also shows greater stability (McLaren and Michie, 1954; Reeve and Robertson, 1953; Dickerson, 1954) against both environmental and genetic disturbances.

The explanation of heterosis is still incomplete (Parsons and Bodmer, 1961), but one possibility is that it depends on "overdominance," the tendency for the heterozygous condition to be fitter than either homozygote, perhaps because in outbreeding animals selection must always be dealing most frequently with the heterozygous state. It will therefore tend to favour gene complexes in which the heterozygous condition of each gene has a more viable phenotypic expression than either homozygote. The hybrid individual has a large number of contributory genes in this favourable condition. The second main possibility is that since the phenotypic difference between dominant and recessive is usually quantitative, and since a hybrid has a large number of growth genes in the heterozygous condition, phenotypically displaying the dominant character, the sum of their effects will be outstanding vigour.

A second genetic property in this general class is that the various genes affecting growth prove to have a multiplicative rather than an additive effect; this has been shown for the genes affecting fruit size in the tomato (Dickerson, 1954). The phenomenon may apply to general growth and will tend to

enhance the contributions of individual genes to hybrid vigour. It is possibly a reflection of the multiplicative nature of growth itself (p. 11).

The hybrid vigour syndrome is important to the auxologist because it seems to support the evidence from other sources (p. 26) that its various phenotypic manifestations are functionally linked properties. This does not necessarily demand that there is a one-to-one relationship between each phenotypic property and one particular gene, or that every gene which contributes to them must be acting at the same level of dominance. If the properties are functionally linked then every growth gene is likely to contribute to many if not all of them and every one adds something to the vigour syndrome when its recessive is replaced by the dominant allelomorph. This also carries the implication that a maximal growth rate and a maximal final size are optimal and provides a strong argument on this side of that question (p. 280).

It is clear that there is much of interest in the genetics of growth but here space permits only a consideration of the general mechanism by which growth has come to be controlled genetically. The analysis is most conveniently taken in two stages, the control in microorganisms and the superimposition of further controls in the Metazoa. Even in microorganisms the problem is formidable enough but this need not be unduly daunting since there is scarcely a more fundamentally important problem in biology; the ontogenetic problem, concerning growth, is a brief recapitulation of the phylogenetic problem of the origin and evolution of life.

As a result of intensive work it has been possible to construct tentative maps of the chromosomes, or linkage groups, of some microorganisms, for instance the simple mould, *Neurospora* (Barratt *et al.*, 1954; Weidel, 1952), the bacterium, *E. coli* (Jacob, 1960) and even viruses (Lennox, 1959; Crick *et al.*, 1961). The genes form a linear series, as in the Metazoa. They show the properties of recombination, and of mutation at definite loci; the mutations commonly involve the loss of ability to perform one particular step of a reaction chain and gave rise to the idea of *one gene—one reaction*. Such mutants usually die or at least fail to grow normally, unless supplied with an intermediary substrate lying beyond the mutant lesion in that reaction chain. In some pathways mutants are known for almost every step, four in the pathway of histidine synthesis (Demerec, 1956) and nine in that of arginine synthesis. As many as 100 such loss genes were already known over fifteen years ago (Horowitz *et al.*, 1945), and 1500 by 1950 (Cohen, 1959).

Supposing that each reaction were simply and uniquely represented in this way by one gene, and further that the genes for any particular series of reactions were arranged in linear sequence on a chromosome, this would constitute perhaps the simplest effective organization of the genome. There is some evidence for such a contiguous arrangement of functionally related genes; three separate genes controlling the synthesis of biotin in *Aspergillus* are located within 0.2 Morgans of each other (Roper, 1950), three controlling the synthesis of *p*-aminobenzoic acid in *Neurospora* are close together in linkage-group V

(Barratt *et al.*, 1954) and there are similar groups for the synthesis of nicotinamide, adenosine, pyridoxine and choline (Pontecorvo, 1952). In linkage-group VII of *Neurospora* a locus for nicotinamide synthesis occurs very near to one for the synthesis of tryptophan, from which nicotinamide can be synthesized (p. 308). In *Salmonella* four successive steps in tryptophan synthesis probably are controlled by four contiguous genes, arranged in the same order (Pontecorvo, 1959). In many loci major subunits, or cistrons, are recognized, likewise all concerned in a common metabolic pathway. The classical position effect similarly implies a specific relationship between contiguous genes in the Metazoa. In some pathways it has been shown that genes cannot cooperate if widely separated, on different chromosomes (Haldane, 1954, p. 113), though for some purposes synergism is possible even between genes of different nuclei, in heterokaryote fungal hyphae.

Unfortunately at present only fragments of the genome appear to show this arrangement based on function, and in some places functionally unrelated genes appear to be contiguous. To some extent this may be due to the incompleteness of present maps; regions of a chromosome which do not mutate or cross over, or which control pathways as yet unamenable to genetical study may be completely missing from the map and in consequence this could be a gross distortion of the complete picture. Secondly, although biochemists know the order of reactions within certain particular pathways these do not yet cover the whole of metabolism. Moreover, while the theoretically ideal arrangement of genes controlling the reactions within a particular pathway may be well known, the arrangement between the groups of genes for different pathways is more uncertain, so that it is not easy to decide how nearly the known fragments of the gene map do fit into an ideal functional arrangement. They may do so better than is apparent at present; in any case, as Pontecorvo (1959) has pointed out, widely separated genes may become approximated for functional purposes, during interphase, owing to the great extensibility and mobility of the chromosomes. This is another example of the importance of the interplay between topographical and morphological relationships; the mitochondria (p. 335) have even greater freedom from morphological trammels.

There are further facts which seem to imply that the organization of the genes is not so simple as the ideal envisaged. As in the Metazoa, genes are often pleiotropic and, reciprocally, more than one locus may control one reaction, so that the one-to-one relationship may be very much obscured. No fewer than 9, 7, 6, and 12 genes, respectively, affect the four steps in the pathway of histidine synthesis. A possible explanation of this would be that a large number of loci must cooperate in the synthesis of the enzymes which catalyse each of the four steps in question: all would therefore control that step, but only indirectly. At present it is more usual to believe that one gene controls the complete synthesis of the molecule of one enzyme, for instance of the transferases (p. 303), and of inducible enzymes (p. 208), and the aphorism is usually expressed "one gene—one enzyme" (Beadle 1945) rather than "one gene—one reaction." On

this view the more probable explanation of the fact that several loci affect each step of a pathway of biosynthesis is that several transferases cooperate in the net transfer of each particular radical, and in fact this was seen (p. 313) to be true. Moreover, each of these transferases is active in a large number of other steps in the whole field of biosynthesis and therefore its determinant gene would be expected to show apparent pleiotropic behaviour. The one gene—one enzyme theory therefore is logically compatible with this kind of complexity. The crucial question is whether typically each locus does in fact determine the biosynthesis of precisely one complete enzyme.

This is not unreasonable in theory, supposing that the enzyme synthesis is of the template type (p. 204), a gene holding the complete code for the copying of a particular enzyme molecule. In fact the template theory is generally favoured for this rather critical part of biosynthesis, whereas it does not seem to be required for the synthesis of the small constituent molecules, the individual amino acids; this is considered to be the work of the transferase enzymes themselves after they are synthesized and released into action. A template mechanism should be possible with no more apparatus (p. 205) than the gene code itself, together with a supply of energy from ATP and other nucleotide pyrophosphates present in the supernatant of the nucleus or of the cytoplasm. Pontecorvo (1959) has suggested that the individual mutational sites (mutons) comprising a gene are each responsible for the martalling in order of one amino acid of the enzyme molecule, directly or via an RNA template. The mutation causing sickle-cell anaemia changes only the a.a. inserted at one site on the haemoglobin template.

Most of the transferases (p. 303), and therefore most of the enzymes required for biosynthesis, contain also a non-protein coenzyme, of the B-vitamin group. As already indicated, these each depend for their synthesis on the enzymes templated from a number of loci. Although some of these loci are contiguous, there may be a considerable number also scattered throughout the genome. The synthesis of individual amino acids likewise requires a considerable number of loci, so that there is the sharp and paradoxical contrast between the postulated single-locus synthesis of the large protein apo-enzyme molecule and the multi-locus determination of each of the constituent amino acids, and of the coenzyme. This paradox may be resolved, without detriment to the template theory of gene action, by the above assumption that the loci found necessary for the synthesis of an a.a. or of a coenzyme include all the loci which synthesize the enzymes used as transferases in its synthesis. When tested by the orthodox type of experiment a locus is likely to appear necessary for the synthesis not only of its specific enzyme but also of any small molecule subsequently synthesized in part by that enzyme. It is often difficult in practice to distinguish between direct and more indirect effects of the action of a gene.

On this theory, the picture of biosynthesis in microorganisms is one of template synthesis of all essential enzymes, by the genetic information carried on the chromosomes, but of enzyme-catalysed synthesis of all other molecules,

including the units from which the enzymes themselves are built. Logically these units represent the first stage of biosynthesis but equally certainly they cannot be synthesized without the necessary enzymes—an important variant of the egg-and-hen problem.

The enzymes may be templated directly on the chromosomal genes, or alternatively RNA facsimiles of the gene, with templating power, are passed into the cytoplasm (Spiegelman, 1948; Leslie, 1961); it is probable that most cytoplasmic enzymes are synthesized *in situ*. It is generally supposed that non-enzymic, structural proteins also are synthesized on templates, so that the aphorism might be further modified to "one gene—one protein" or "one cistron—one polypeptide" (Woodward, 1959). However, template synthesis may cover only the primary synthesis of polypeptide chains, or at most the secondary folding of these. The genetic control of tertiary synthesis (Chapter 13), the formation of cell organelles (Chapter 11) and the control of cell division (Chapter 21) remain to be explained. Organelles, in general, may be self-replicating but, as in the case of the kappa particle (p. 143), they are ultimately genetically determined. Consequently there may be genes with growth functions other than as templates for polypeptide chains, quite apart from the further possibility of genes concerned in physiological functions other than growth. Even for microorganisms, therefore, there may be no simple aphorism which is a complete genetic definition; even so there are grounds for reasonable optimism that the genetic information is coded in some relatively simple way.

In the Metazoa, as already emphasized, the genetic information must include instructions also for morphogenesis at the multicellular level, that is for a four-dimensional change in form, as well as for the elaboration of their hierarchy of controls of growth, from the systemic to the molecular level. The simplest logical representation of the genetic information in the Metazoa, if not the most functional, as an evolutionary development, would be one (p. 385) containing genes specific for each level of control, each stage of ontogenesis and each part of the body, as well as the pristine enzyme- or protein-specific loci.

Some evidence of genes specific to the hormonal level of control has already been given (p. 385). In addition many loci are known which are highly specific to other levels. The giant mutant of *Drosophila* causes the larva to continue feeding for a longer time than usual, and to grow unusually large for that systemic reason (J. Needham, 1942, p. 262). Other factors acting on total growth rate, or on size, are known; certain of the mutant phenotypes which can be mimicked by some simple change in a single external condition, producing what are called *phenocopies*, probably also are due to genes which act at the highest level, on the process as a whole. Tissues from some lethal mutants will grow in a normal host (Hadorn, 1948, 1961), so that their genetic lesion is certainly at a level above that of the individual cell.

Some mutant genes control differential growth between the parts of the body (Huxley, 1932; Sinnott and Dunn, 1935; Hadorn, 1948). In *Drosophila* the "chubby" mutant is shorter but broader than normal and "rudimentary 12"

has short wings but normal body growth. Here control is at the systemic, or at the organ, level. The "grey lethal" mutant of mice shows defects of almost all the bones, that is of a whole and diffuse organ system, and many other organ-specific genes are known. Some genes act mainly on cell proliferation (Goldschmidt, 1938, p. 212), others mainly on cell growth (Gruneberg, 1948). A yeast mutant studied by Mallya and Subramanian (1949) affected the rate of proliferation of the cells.

In the Metazoa, as in microorganisms, genes are known which control the synthesis of a particular metabolite. In some cases genetic differences in growth rate are correlated with differences in a specific substance, for instance in glutathione content (J. Needham, 1942, p. 426; Dickerson, 1954). Many genes affecting pigment synthesis are known, both in vertebrates and in insects; these may affect the colour of the whole body or they may act locally in space also, on eye colour. Finally the mutant gene causing sickle-cell anaemia merely changes a single one of the 300 amino acids of the haemoglobin molecule. It must be borne in mind that genes sometimes appear specific to a particular level merely because of incompleteness of knowledge, but it seems clear that there is a considerable degree of specificity of genes to particular levels.

It seems probable that many site-specific genes, acting only in one part of the body, are phenotypically or epigenetically rather than simply genetically controlled. The phenomenon is one of local genetic losses or suppressions, for the most part, though sometimes of local activation of dormant genes. In the classical case of the nematode, *Ascaris*, and in other animals, genes and indeed whole sections of chromosomes are progressively thrown out of the nucleus of successive generations of all cells except those of the germ line (Goldschmidt, 1937). This is called chromatin diminution and some animals eliminate whole chromosomes (Darlington, 1932; Wright, 1941). The losses vary in nature in the different tissues of *Ascaris*; presumably active genes persist only in those tissues where their activity is required. There is some evidence of such regional differentiation in the Protozoa also, between the nodes of the macronucleus of the ciliate *Stentor* (Weisz, 1949), each node controlling the development specifically of the body region around it. The macronucleus of ciliates is highly polyploid and a regional differentiation between sets of chromosomes, initially identical, seems the most likely basis for nodal differentiation; it also is compatible with what may be expected to have happened during evolution. However, this regional differentiation in the nodes of *Stentor* has been questioned by other workers: Tartar (1956) found that one node can regenerate a whole nucleus. If the differentiation does occur it is a regularly repeated ontogenetic process, no doubt, as in the Metazoa.

There appears to be a progressive, regional differentiation between nuclei even during the ontogenesis of such animals as the Amphibia, which show no gross nuclear changes (King and Briggs, 1955; Fischberg *et al.*, 1958). If transplanted, nuclei retain their existing stage of differentiation. These changes help to explain the irreversibility of tissue differentiation and the severe restriction on

metaplasia, or change of histological type, even in metazoa as primitive as the Porifera. The way in which regional differentiation of the genome is effected in the Amphibia and most other metazoa remains virtually unknown, though it would be compatible with the kind of progressive and reciprocal interaction between nucleus and cytoplasm postulated most recently and comprehensively by Waddington (1948, 1957). From the outset there is a progressive differential partitioning of the cytoplasm between daughter cells, and at each stage this may cause differential changes in the initially identical daughter nuclei. This in turn confers new heterogeneity on the cytoplasm in each cell, leading to further segregations between the cytoplasms of the two daughters of the next division. It is worth emphasizing that this epigenetic process can be regarded as the streamlined codification of the underlying phylogenetic process of division of labour, which restricted spatially the expression of the pristine genes rather than created new ones.

This is not the complete explanation of regional differentiation and site-specific genes. As shown below, losses of portions of the genome already in the zygote have differential effects on the different germ layers, ecto-, meso- and endoderm and other genes or groups of genes are specific to particular organs. Hadorn (1961) describes a large number of such site-specific genes; for instance, a mouse mutant suffers degeneration of the cartilage but of no other tissue. Some mutants affect all bones, but the "creeper" gene of fowls and the "streamlined" gene of pigs affect only the limb bones, while human brachydactyly affects only the second phalanx of the second digit! It is possible that these genes simply work through the regional "screening" already considered but it remains possible that they are genetically instructed to act in particular sites, and are not environmentally constrained to do so. This is the usual nature/nurture problem.

There is no absolute separation of spatial from temporal changes during metazoan development and in principle some genes might be stage-specific as an indirect result of this progressive regional differentiation. It seems even more plausible that site-specificity may sometimes result indirectly from stage-specificity. Certainly some genes are stage-specific (Hadorn, 1961), and often very sharply so. For instance the "creeper" factor in chickens manifests itself at an early stage, while subsequent growth is as normal as it could be in an embryo already so abnormal (Goldschmidt, 1938, p. 212). Most stage-specific genes act at a few well-defined stages which are special crises of development (Hadorn, l.c.); some genes in fact act at more than one of these. Some genes, at least, can be regarded as being now primarily stage-specific in their expression. Few single genes have distinct effects before gastrulation (Runstrom and Gustafson, 1951), though substantial losses of chromosomal material do cause earlier abnormalities (Poulson, 1945).

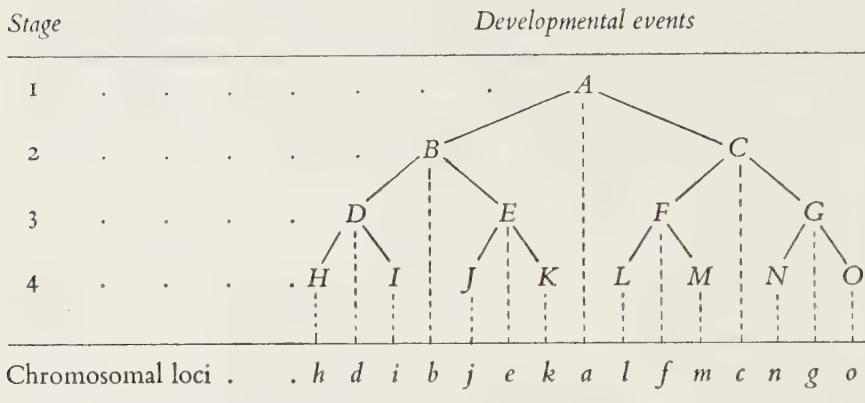
The study of development in individuals of *Drosophila* bearing relatively large chromosomal deletions (Poulson, l.c.) has added much to knowledge of this and related aspects of epigenetics, though interpretation of the results presents some difficulties. A relatively small fraction of the whole genome, the

X (sex) chromosome, contains genes which collectively influence the complete development of the body, and loss of this chromosome, in the "nullo X" mutant, arrests development virtually at the outset. Nullo X zygotes show incipient nuclear division to give a collection of nuclei anteriorly, while posteriorly there is cytoplasmic cleavage, devoid of nuclei (p. 344). Development goes no further, and therefore this one small chromosome can control the entire process of early development. It seems likely that the loss of any other chromosome piece of this size might have an effect of at least equal magnitude, with early arrest, since the sex chromosomes in fact are usually the least important for somatic development.

Loss of only the  $X_L$  half of the  $X$ -chromosome allows development to proceed as far as the formation of the blastoderm. Loss of only the smaller "facet," or "notch," regions of the chromosome, containing about 45 of the visible transverse bands or chromomeres, permits development as far as the differentiation between ecto-, meso- and endodermal tissues but there is a disproportionate amount of neural tissue relative to the general ectoderm, while both meso- and endoderm show even poorer further differentiation and growth. The "white" deficiency, of about the same size, allows fairly normal further development of ectodermal tissues but not of meso- or endodermal structures, and the insect lives to the stage of hatching. The apparent implication is that any large section of a chromosome effectively controls the general development of the whole body from the outset, while any section of progressively smaller size controls only proportionately smaller details, of successively later stages of development. The earliest stages therefore would seem to require the synergism of all genes, and successive stages that of a progressively shorter series of loci, until the final details would presumably depend on single genes, in isolation. The facts also might seem to imply that at the outset there are no great differences in properties between any sections of about the same size, throughout the genome, so that any differentiation between sections would have to develop epigenetically, during ontogenesis. The nuclear differentiation which accompanies the regional differentiation of the body would be part of this.

However, this picture is not easily reconciled with the classical view that the genes are always fully "informed" and that the genome must have the same high degree of differentiation between loci at first as later: their rate of activity may change but not the quality of their action. There is an alternative picture more consistent with this and other aspects. Hadorn (1948) favours the view, for which evidence was given above, that stage-specific action depends on individual genes, sharply localized in the genome and he has suggested that there are early-acting genes scattered throughout the genome; consequently any large section of a chromosome would contain at least one of these, and there would be a high probability of any small section having none. It seems a necessary corollary that the abundance, per unit length of chromosome, of genes specific to each particular stage increases with successive stages of the developmental process. The representation, at gene loci  $a, b, c, \dots$  on the chromosomes,

of the control of events  $A, B, C, \dots$ , occurring in sequence at stages 1, 2, 3, . . . of development therefore conceivably is as follows—



If the projection were as regular as this, virtually any section of a particular large size would have the same number of genes specific to each of the different stages, but clearly there should be lucky small deletions and mutations, at such loci as *a*, which would affect the earliest stages of development, and on the other hand large deletions such as *h-k* which affect only later stages, from 2 onwards. The plausibility of this idea increases with the value which is accepted for the total number of genes, that is with the extent to which the phenomenon has a statistical basis, capable of dominating the specificity of individual genes. Some of the events  $A, B, C, \dots$  could be site-specific and some both site- and stage-specific, as already suggested.

There is some evidence from the work on *Drosophila* (Poulson, 1945) for this kind of sharp localization in the genome of early-acting genes. While loss of the  $X_L$  half of the  $X$  chromosome has the limited effect described above, that of the other,  $X_R$ , half has almost as much effect as a loss of the whole chromosome, and must contain the early-acting genes of the chromosome. Evidence that a large number of genes are quite specific to particular developmental stages was obtained by selecting, from a single stock of mice, one line for rapid, and another for slow early growth; this left both lines still with an identical mean growth rate in later stages (Dickerson, 1954). The way in which the action of a gene is restricted to a particular stage may be somewhat similar to that for site restriction, that is to say a series of actions between nucleus and cytoplasm may be essential for the ultimate expression of each gene and the length of the series may vary according to the stage to which it is specific.

If, as seems very plausible, stage- and site-specificity in the Metazoa have in general evolved simply by appropriate restrictions on the expression of pre-existing genes, then it is to be expected that the genes so affected lie scattered through the genome, as in the scheme above. The pattern of their distribution

may be functionally significant but it is perhaps premature to enquire further into this; it promises to be a useful problem for the future. Like the sequence of amino acids in a protein it may be highly aperiodic but not necessarily chaotic.

There remains the problem of the evolution of the genetic representation of level-specific controls in the Metazoa. Did this involve the creation of new genes or merely the modification of the pristine genes of a unicellular stage? In the latter event was the modification similar to that by which other genes became primarily site- or stage-specific? The creation of completely new genetic information seems highly improbable, if not meaningless, to the student of evolution. Every advance is built on existing, well-established foundations, and is dependent on the ability of these to change. The nearest feasible approximation to the acquisition of new genes would be that an existing piece of the genome should be replicated along the axis of the chromosome, the redundant piece then being at leisure to produce by mutation modified genetic instructions, relatively independent of those of the parent piece. This is only a particular expression of the intuitively demanded aphorism "every gene from a gene." As already indicated it is also the most extreme case: at the other extreme a relatively slight modification of a well established gene might be adequate to convert it to the control of growth in a metazoan, even at the systemic level.

Notwithstanding the degree of uncertainty on this question, it is possible to visualize the mode of evolution of level-specific controls in relation to site- and stage-specificity, and to make some inference from this about their genetic basis. The metazoa probably evolved not from complex but from relatively simple unicellular organisms, since anything with the high degree of differentiation and efficiency of a typical ciliate could only be hampered by cellular associations as intimate as those found in metazoan tissues. Even so, the subsequent division of labour among the associated cells must have involved some loss of properties by each cell, some simplification of differentiation, which indeed was probably progressive, individual cell types becoming more and more specialized. This was the phylogenetic origin of the site- and stage-specificity of ontogenesis, though ontogenesis today begins in a cell, the ovum, which overtly is maximally dedifferentiated, and not in a maximally differentiated cell. Protozoa can dedifferentiate, and do so in connexion with each act of fission, so that the ovum probably can be regarded as simply a specialization of this power. Moreover, during ontogenesis in the Metazoa, differentiation has become simplified to a single sequence, spread over the generations of cell proliferation, as also in a population of bacteria (p. 116); however, as already remarked (p. 119), cells *in vitro* still show traces of de- and re-differentiation in every cell cycle, and this may be true *in vivo*, also.

Initially site-specificity could only mean restriction to one cell type or another. These cell types or germ layers, were distributed uniformly throughout the body of the primitive metazoan, as in the Porifera still, so that any small fragment was virtually identical with any other. Apart from a vaguely defined

integration into a sponge "person," therefore, the only level of control above that of the individual cell concerned the simplest and most local of cellular interactions. Ontogenetic development was correspondingly simple so that stage-specificity also had as yet little meaning. It would therefore seem that stage- and site-specificity evolved in parallel with organization, and with the growth-controls specific to particular levels of organization.

The initial wide distribution of all essential tissues throughout the body persists with relatively little change in metazoa as highly evolved as the platyhelminths, though the integration of their nervous system has already established a primitive systemic level of control, which is admirably demonstrated in the process of regeneration. Another systemic control has been established in these animals, in the form of migrant "neoblast" cells, which remain active in annelids although these have also perfected the most important adaptation for systemic control, a fluid circulatory system. As soon as this was developed, each tissue could become segregated into a compact organ, supplied via the circulation, instead of having to make its own contacts throughout the body. The segregation effected new levels of organization, between the systemic and the tissue levels, and the genetic control of the various levels may be envisaged as having evolved in the same, somewhat complex order.

The nature of site-specificity also changed with these changes in organization, and ontogenesis acquired new stages. Moreover genes which had by now become site-specific to particular organs also became the determinant genes for new controls at the systemic and other levels, as for instance, through the production of hormones by localized endocrine organs. Consequently level-specific genes may be related to those for site- and stage-specificity in more direct ways, as well as by concomitant evolution.

As in the evolution of biological organization in general (p. 3), metazoan evolution involved the replacement of a relative micro-heterogeneity, between neighbouring cell types, by a macro-heterogeneity between organs; since average body size increased progressively during evolution this heterogeneity acquired an ever coarser grade of size.

This demanded a corresponding increase in macro-heterogeneity of the integrating systems, the centralized nervous system as compared with the simple nerve net of the coelenterates, and the discrete hormonal system as compared with the diffuse cell-to-cell interaction of the lower Metazoa. During ontogenesis this phylogenetic process is recapitulated in greatly modified form. The segregation of organs runs ahead of their visible differentiation. Their chemical differentiation, however, shows a progressive focusing (Fig. 27.1) as in phylogensis (Ebert, 1958).

To summarize: in microorganisms the genetic information may initially have been restricted to templates specifying the essential proteins, which in turn control the synthesis of other materials, including their own constituent amino acids. The genes themselves are self-replicating. It seems probable that separate genetic instructions were then required for synthesis at higher levels, to

form skeletal materials, cell organelles and so on. Other independent genes may have become necessary to control cell division, and possibly also to determine the properties of interaction between cells in the metazoan state. Much of the further organization of the Metazoa may depend simply on a restriction of gene action to particular sites. The pristine, protein-specific genes are probably arranged in patterns appropriate to the subsequent co-operation of these proteins, in further syntheses. Little is known of the arrangement of other genes within the genome, or how this was determined, during evolution. In metazoa genes have been recognized which are specific to particular sites in the body, to specific stages of development or to particular levels in the control of growth, but it is not yet certain how they are related, either spatially or in history, to the pristine genes controlling biosynthesis.

There are probably few which do not affect growth in some significant way and the genome contains the codified evolutionary history of growth. The study of epigenetics, that is of the way the genotype is translated into the phenotype, has already thrown much light on the code, but naturally enough it is a difficult one to break in the Metazoa. The genetical study of microorganisms has produced a much deeper knowledge of growth control at the intracellular level. This is the meeting ground of a number of special disciplines, with penetrating techniques.

## CHAPTER 25

### *External Factors and the Control of Growth*

GOODRICH (1912, p. 33; 1924) and others have stressed the intimate interdependence of genetic constitution and environment, of nature and nurture, particularly in the development of an organism. The normal phenotype is the result of a continuous interaction between the genotype and the environment in which it develops, and growth measurements can be meaningless if the nature of the environment is not specified. The intimacy of the interrelationship is illustrated by food, which must be considered as initially an environmental factor, while later it becomes fabric, and some of it genetic material. For some purposes it may be useful to recognize a special internal environment, that of the body fluids, but not all animals have such a definite internal medium; in any case body fluids, like tissues, are genetically controlled and the main distinction is between internal factors collectively and external factors.

The relevant components of the external environment may be classified as biotic, the influence of other living organisms, and abiotic—inorganic materials and physical forces. The distinctions are not absolute but they are almost as valid practically as logically. Biotic factors will be considered first since they are “nearer home” to the growing organism itself: indeed they include its mother’s uterus and even her ovary, relationships approximating to the intimacy of tissues within a single body.

#### **25.1. Biotic Factors**

The most intimate biotic factors are those concerned with the provision of yolk, in oviparous animals, and with placental supply in viviparous ones. Yolk is deposited under the control of the maternal metabolism and other less evident properties of the egg cytoplasm also are maternally determined, for instance dextrality or sinistrality in the snail, *Limnaea*, and the incidence of diapause in insects (p. 426). The growth rate, fecundity and longevity of certain rotifers varies with the age of their mother, who presumably determines these properties through the egg cytoplasm. Larvae of the mealworm, *Tribolium*, from eggs of mothers fed 60 per cent extracted (white) flour grow faster than those from mothers eating 85 per cent extracted (brown) flour (Reynolds, 1942). In birds, egg size, that is mass of yolk, is more important than genetic constitution in determining hatching size (J. Needham, 1942, p. 427), and prenatal growth rate. After hatching, external environmental factors may modify this initial relationship (Dickerson, 1954). In fish (Gray, 1929a), and tunicates (Berrill, 1945) also, egg size determines the final size of the embryo. By transplantation of yolk

between eggs of different breeds of chick, Ryle (1957) demonstrated some effect of yolk quality on the growth of the embryo; curiously enough these effects were clearly detectable only after hatching.

The effect, on the growth of larval *Tribolium*, of feeding thyroid to their mothers is cumulative over several generations (Schneider 1940) and this trans-generational effect is seen also in mammals, via placental transmission. Marshak (1936) thought that hybrid vigour depended on a maternal, cytoplasmically transmitted factor, but this seems to be mainly genetical (p. 386). However, Borisenko (1939, 1941) found that the offspring of two parents of the same pure line showed hybrid vigour if these parents had been reared in different environments, so that there may be at least a subsidiary influence of the maternal environment. Pure-line zygotes implanted into hybrid mothers also grow more rapidly than normal (Venge, 1953).

In some insects a poor maternal diet causes the eggs to develop rapidly without diapause (Simmonds, 1948) and a generous diet has the reverse effect. There are parallel phenomena in plants. Ageing of the parent insect also causes her eggs to develop more slowly, with diapause, and a raised external temperature more rapidly, and without diapause.

Viviparity occurs sporadically throughout the animal kingdom but there is little detailed knowledge of any but that of the mammals, and of a few of the lower vertebrates. Details of placental transmission and general physiology need not be considered here (J. Needham, 1931, 1942). Placentae vary considerably in structure and efficiency but it is not clear that the growth rate of the foetus depends critically on this. The haemochorionic placenta of man is among the most efficient on most counts, but the human foetus grows more slowly than that of any other mammal of the same size. Again mammalian foetuses grow more slowly than bird embryos (J. Needham, 1931) but this may reflect taxonomic differences and differences in biological desiderata rather than differences in efficiency of the prenatal mode of nutrition. Selection for ability to grow satisfactorily *in utero* presumably has necessarily been so severe in the past that only very drastic treatment of a female mammal can adversely affect the growth rate of her foetus. The foetus aborts if the mother receives less than 5 per cent of protein in her diet, but if it does develop then it develops normally (J. Needham, 1942, p. 77). Indeed the growth of a young mammal between birth and weaning may be more dependent on the mother's characteristic of milk yield, than is the prenatal growth on her uterine conditions (Dickerson, 1954).

Prenatal influences in mammals are considerable, nevertheless, and the birth weight of babies is related to the mother's basal metabolic rate (B.M.R.). Those from mothers with a low BMR grow slowly, though they soon compensate, by a higher growth rate after birth (Sontag *et al.*, 1944), so that such prenatal influences are not necessarily permanent or important. In polytokous mammals the size  $y$  of the foetus (and of the whole litter) at birth is quantitatively related to the number,  $x$ , in the litter by the type of relation  $y = ax^b$ , where  $a, b$  are constants (Crozier, 1939). The relation is non-linear, perhaps because the

competition between foetuses is indirect (p. 356). In a large litter, chemical composition is normal, even if the weight of the individual is low. Some effects of the prenatal environment are more permanent and serious, and qualitative as well as quantitative abnormalities can result from pathological intra-uterine conditions, as when the mother is affected by German measles or by syphilis. However, in a sense pathogenic organisms are part of the external environment, at least of the foetus. Since the prenatal period of mammals is the period of rapid growth, only the most powerful selection for the maximal independence of environmental vagaries could make viviparity a safe practice.

Protection of the foetus is of paramount importance but protection of the mother also has been a necessity of selective value. It is therefore interesting to find that the birth weight of the foal of a Shetland mare by a Shire stallion is little bigger than a pure Shetland foal, but that of the reciprocal cross is much larger; this is not to deny the importance of genetic factors of course, and the size of young rabbits from a particular doe is related to the size of the father (Beatty, 1956). Again the number of fertile ova which become implanted in a polytokous mammal is related to her body-size, and increases with her age in those cases where she grows after maturity (Venge, 1950).

The mother is able to adjust to the demands of her foetuses and a ewe carrying twin lambs eats more than one carrying a singleton (Tribe, 1950). From the study of placental physiology it is clear that much positive provision is shown by the pregnant female. The placenta grows ahead of the foetus, just as the chick's embryonic membranes do (J. Needham, 1942, p. 79). Birth size and gestation time in the various species of mammals are related to adult size rather closely, by a log/log relationship (J. Needham, 1931, p. 470). A similar relationship is maintained by birds, for hatching size, which depends also on egg size, that is on food supply. In mammals also, therefore, the foetal food supply may be related to a power function of maternal body size. The growth of the foetus necessarily is under the control of the prevailing maternal hormone, progesterone, and the blastocyst will not grow *in vitro* or in the uterus of a spayed female (J. Needham, 1942, p. 84).

Transgenerational effects are probably more common in mammals than in non viviparous animals for evident reasons. Effects of altitude, darkness and laboratory conditions on growth often are shown only in the next generation (Brody, 1945, p. 545; Schneider, 1940; Luce-Clausen and Brown, 1939) and often continue over several generations.

Postnatal influences by parents may be considered here. In mammals these can be particularly important up to the time of weaning. Mice suckled by female rats grow more rapidly than normal for simple quantitative reasons (Parkes, 1928). Rutman (1950, 1951) made the very interesting discovery that slowly growing strains of rats when suckled by mothers of fast-growing strains increased their rate of methionine uptake by the liver, a measure of protein synthesis; a growth-promoting factor therefore is contained in the milk—in higher concentration than in the milk of the slowly growing strain,

Among other fairly direct influences by individuals of the same species are those of "ectohormones" or "pherohormones" secreted externally and ingested by other individuals (Karlson and Lüscher, 1959; Micklem, 1959). The determination of caste in termites depends largely on this, as the method of control for growth and differentiation (Lüscher, 1960). In the Hymenoptera such agents control sexual maturation but here early growth and caste determination depend more simply on diet, an external factor but in this case selected by other individuals of the species.

A young larva of the worm *Bonellia*, settling on the proboscis of an older female individual has its growth accelerated, but also severely curtailed because maturation also is accelerated, by a steroid secretion of the proboscis (Carlisle, 1957); it develops, in consequence, as a dwarf male. Development free of this contact is slow and protracted and produces a female, so that sex as well as general growth is controlled by the transhormone. Other cases of growth and sex control by such contacts are known, in *Crepidula*, the slipper limpet (Baker, 1926), in the oyster, and in barnacles, and similar humoral influences are suspected in other cases. Association of a *Crepidula* larva with an adult improves its nutrition and growth rate and leads to differentiation as a male (Coe, 1948), but nutrition may not be the only controlling factor.

Many cases of homotypic conditioning are relevant here. This is the ability of individuals of the same species of certain aquatic animals to condition the medium for each other (Allee, 1934; Allee *et al.*, 1949). Under experimental conditions, at least, it is rather rare for a single individual microorganism or a small metazoan to grow well immediately it is isolated into a new medium. It may effect its own conditioning in time, but the conditioning activities of a group of individuals summate—or even multiplicate. The lag period is proportionately shortened (Hinshelwood, 1946). In many cases experimental media are somewhat abnormal, if not grossly unsuitable, and the conditioning may involve detoxication of particular deleterious chemicals in the medium, the buffering of  $H^+$  or  $OH^-$  ions, and so on. Conditioning may vary from such prosaic effects to something much more subtle. Microorganisms secrete considerable organic matter into the medium (Gale, 1953) and in some instances transhormones may be involved, their biological function being to encourage growth only when the *biological* environment is propitious. There is no good evidence that trout produce specific conditioning substances (M. E. Brown, 1957), but Allee *et al.* (1940) found a potent agent of this kind produced by goldfish. Its action was depressed by excretory products. Even inorganic systems sometimes require similar phenomena, for example the nucleation of crystals.

In terrestrial poikilotherms grouping may speed growth simply by increasing the temperature or the humidity. Rodents and other terrestrial homoiotherms also grow better in groups than when isolated. In these cases the effect must be mainly neurological or neuroendocrinological. That there may be a large neurological or psychological factor in these phenomena is shown further by the work of Petrusewicz (1959) on mice. Here almost any experimental change

in numbers, whether an increase or a decrease, caused an increase in growth and reproduction rate. His mice showed, in addition, spontaneous rhythms in growth and reproduction due largely to population density interacting with a second, out-of-phase factor, in the manner typical of oscillatory responses (p. 19). Induced growth and proliferation were greater in large than in small colonies while the reverse was true of the spontaneous increase in numbers, of course.

Other influences between individuals may be growth-depressant, at least on the weaker brethren, and these also may be mainly of the more subtle, neurological type, working via the neurohumoral system and not via the gut or the gills, though appetite is often an important mediator of the effect (Brown, 1946). Fully fed hens will resume eating in the presence of added individuals and ewes show the same behaviour, just as children do at school meals, or at parties, compared with their performance at home. The psychological factor of novelty is a further complication here. Overcrowding depresses the appetite of trout fry even if fed *ad libitum*, while undercrowding leaves it fickle and desultory, as in the cases above (Brown, *l.c.*). The ideal is 11 fry per litre. Rats and mice thrive best at a density of 2–4 per cage. The optimal density is greater for young than for older tadpoles, and available food naturally could be the limiting factor here, but 8 individuals of the beetle *Ptinus tectus* require 100 times as much food as one isolated individual (Wigglesworth, 1954*b*; p. 48): they eat more yet grow less. Davenport (1899) already could cite many examples of these interesting effects, which vary greatly in detail between different animals. The accumulation of excreta or other toxic materials can inhibit growth but this was discounted in most of the cases examined. Queen ants in some way depress the growth of larvae (Brian and Carr, 1960), possibly by the "queen substance" transhormone which depresses sexual development in the workers of some hymenoptera.

In some cases at least no inhibitory substances are secreted (Brown, 1957), for instance tadpoles suffer from crowding just as much in running water as in still water (Allee, 1934), and nothing significant is exhausted, since new cultures in filtered old media from overcrowded cultures, may grow as well as in fresh media. Some workers, however, have detected specific inhibitory factors. That of tadpoles (Rose, 1959; West, 1961) is heat-labile and non-dialysable, and is probably a skin secretion (West, 1961). This helps to explain why it is not relieved, but may be exacerbated by increased food supply. In this case, in contrast to earlier results quoted above, changing the water did prevent the inhibition. Certainly it is true that individuals which are small owing to growth inhibition will grow well if removed from the presence of their rapidly growing comrades (Brown, 1946), but if these smaller members of a group are isolated they produce the same size-scatter as the original large group; the largest grow relatively larger and the smallest relatively smaller. For some animals it is the amount of *lebensraum* which is critical, irrespective of the presence of other individuals (Davenport, 1899).

Effects of crowding on differentiation also are well known. Crowding of Cladocera causes fat deposition, that is a change of metabolism, as well as growth inhibition (Smith, 1915). The production of the migratory phase of locusts depends, in part at least, on crowding and again involves metabolic changes. Other effects, indirectly connected with growth, are observed. The female corn weevil, *Calandra*, chooses fewer grains in which to lay her eggs if the colony is crowded (Allee, 1934). Crowding affects sex in Cladocera and in other entomostracan crustaceans: an isolated individual may become hermaphrodite and capable of self-fertilization (Longhurst, 1954).

Heterotypic conditioning has been described in many cases (Allee, 1934), that is growth stimulation due to the presence of individuals of other species. Between microorganisms the effect is directly at the metabolic level (Burrows, 1942). Aquatic molluses appear to promote the growth of fish. It is not always easy to distinguish between such types of growth promotion and that due to symbiosis. Many animal symbionts are absolutely dependent on their partners (Caullery, 1952; Trager 1960), even for survival, and certainly for growth. This field is much too vast for any survey here, and Caullery's book should be consulted. Virtually all cases which are significant for growth are associations between an animal and an autotrophe; the latter provides vitamins at least, and, in the most specialized cases, carbohydrate or other fuel, from refractory materials such as cellulose, chitin, wool and wax. Some effects of the rumen micro-organisms of artiodactyls have been mentioned earlier (p. 235). In some cases (Yonge, 1944) symbiosis may benefit the animal mainly by removing waste-products such as  $\text{CO}_2$  and  $\text{NH}_3$ . Some animals can develop a different gut fauna to deal with a new diet—a process known as "refection." Cophrophagy, the consumption of the animal's own faeces, may help in refection; it also helps in a more complete recovery of the vitamins and other products of microbial activity, particularly when this occurs in the large intestine, or its caecum, where digestion and absorption by the host are limited.

Heterotypic inhibition of growth by individuals of another species also is common. This also may range from some very simple type of competition for food, or the production of a simple toxic metabolite, to the production of complex and specific antibiotics (Lucas, 1947; Waksman, 1947). It is the taxonomic selectivity of antibiotics which makes them so useful clinically. Penicillin and other antibiotics of fungal origin are specific to particular types of bacteria. Streptomycin is effective against many of the bacteria not inhibited by penicillin and the two together constitute an effective antiseptic when added to cell cultures *in vitro*, to tissue homogenates used to study enzyme reactions, and so on.

Most antibiotics are growth-inhibitory or auxostatic (Brian, 1947) on their target-organism rather than completely lethal or biocidal, and growth arrest is usually a sufficient defence. The victims are most sensitive, however, when growing most rapidly, and may then undergo lysis in fact, whereas when already growing slowly, the effect is merely to arrest activity (biostatic effect). Antibiotics thus control the growth of organisms likely to compete for food in a

natural environment. Some are oligopeptides (p. 287) often containing at least one amino acid in the form of the D-optical isomer, or in some other unnatural form, and these may act as metabolic antagonists of the corresponding natural amino acids of the victim (Simmonds *et al.*, 1951; Dalgleish and Todd, 1949). Actinomycin has threonyl-L-proyl-D-valyl-N-methylvaline. They vary very much in chemical structure but probably all are antimetabolites. Penicillin contains the thienyl ring, a potential antagonist of the thiazole ring of thiamine and of biotin. Antimetabolites commonly displace the natural analogue on the reactive site of a critical enzyme, and if they form a stable compound they may inactivate the enzyme completely at relatively low concentration. Antibiotics are probably usually of this type since they are active in concentrations too low for any effective mass-action competition. They are all biologically unusual, if not unique, substances (Michael, 1947), and have thrown much light on the action of artificial growth-inhibitors. Most have unsaturated bonds in the molecule and are therefore highly reactive (p. 106). Many are cyclic compounds.

Their mode of action is very variable, and in some cases has been studied in great detail because of the clinical importance of bacteriology in general, and of antiseptics in particular. Most antibiotics are harmless to man and other mammals. Many of them throw light on the basic chemistry of growth; tyrocidin and some others are surface-active and perhaps inhibit growth by a more physical and non-specific action on cell permeability. A considerable number appear to be oxidants since SH-compounds mollify their action (Winterstein and Dutcher, 1949). Gramicidin and aureomycin uncouple phosphorylation from oxidation, in the mitochondria (Colowick and Kaplan, 1951). Aureomycin, terramycin, neomycin and streptomycin are found to inhibit protein synthesis and bacitracin and penicillin inhibit that of the nucleic acids also (Gale, 1953). The first three may actually stimulate NA-synthesis at concentrations which prevent protein synthesis: possibly this removes a normal competition, arising from protein synthesis, for glycine and other potential NA-precursors, but in addition they possibly stabilize RNA as chloromycin does (Dagley *et al.*, 1962). These three antibiotics also permit incorporation of glutamic acid (GA) into existing protein at concentrations which completely prevent protein neoformation. Neomycin inhibits growth completely when its inhibition of protein synthesis is still only 10 per cent, and presumably its action is specific to one or a few particularly important proteins. Growth as a whole is quite often found to be in certain respects more sensitive than particular components of the process (p. 188). With aureomycin and terramycin the degree of growth inhibition is simply proportional to the degree of inhibition of protein synthesis. Penicillin inhibits transpeptidation by GSH (p. 194).

Antibiotics in small quantities have been found to increase the growth rate of children, pigs, amphibia and other animals, even on an already ideal diet, with a full complement of vitamins (Collier, 1952). In most cases they are thought to kill differentially or to inactivate the more deleterious of the gut

flora, though other workers have found an effect on nitrogen metabolism or specifically on haemoglobin synthesis. P. B. Brown *et al.* (1952) found the effect of aureomycin on pigs to be simply on the amount of food consumed, but the differential antibiotic effect seems the most general explanation. Sometimes growth is accelerated in consequence.

Heterotypic inhibition of growth by actual contact is seen in many cases of parasitization. The deleterious gut flora just considered come under this heading. Even ectoparasites may be a serious menace, though frequently there is the complication that dietary deficiency in the host is itself a predisposing factor to lousiness. Ectoparasites, particularly of birds, may depress growth by their irritation, once more by neurological means, as much as by their demand for material. However, internal parasites commonly do act as a serious drain on material resources and they may also secrete toxic materials. The cirripede parasite, *Sacculina*, effectively an internal parasite of *Carcinus*, inhibits growth and regeneration (Cornubert, 1953), probably via an effect on the growth endocrines (p. 379). Reciprocally the host may affect the development of a parasite (Salt, 1941).

In sedentary animals very dramatic effects on growth and form can result from mere mechanical causes. Barnacle larvae settle in such dense colonies that the individuals soon cramp each other by their lateral growth. The only solution (Fig. 25.1) permitting each individual to develop a reasonable frontage on the open water seems to be a rhythmic variation throughout the colony in the amount of growth in height; individuals grow tallest at certain points, roughly evenly spaced, and shortest at points midway between, so that the surface of the colony as a whole becomes maximally increased by folding (Barnes and Powell, 1950). The determination of this interesting pattern of growth is not clear, but it is likely that fortuitous differences in density may determine the initial pattern, which tends to be self-exaggerating. The effect on the form of the individual at different points of the spatial period led in the past to the recognition of a number of spurious taxonomic varieties of the species.

The effects of biotic factors on growth tend to be very sharply black or white, strongly inhibitory or acceleratory. This is natural enough since both the factors themselves and the process on which they are acting are the products of evolution, which is never casual or indecisive. Whether the effect is inhibitory or accelerating it is adaptive for one or both organisms involved in the relationship. In some cases, such as that of the trout fry, the species benefits rather than its every individual; for the species it is advantageous to give more to "him that hath" and it is no serious loss if the weaklings go to the wall. This is also an ideal example of an enhancing mechanism, making its own black and white from a range of greys.

It might reasonably be claimed that the action of biotic factors on growth will teach us more about evolution than about the growth process itself, though there are several notable exceptions. Antibiotics are a group of chemical agents not only each specific to particular components of growth but also to a particular

group of organisms. The information to be obtained from the study of this group is potentially very great. The study of pheromones, of chemical agents which "condition" aqueous media, and of other trans-individual agents also holds a great deal of promise. Maternal-foetal and embryo-yolk relationships

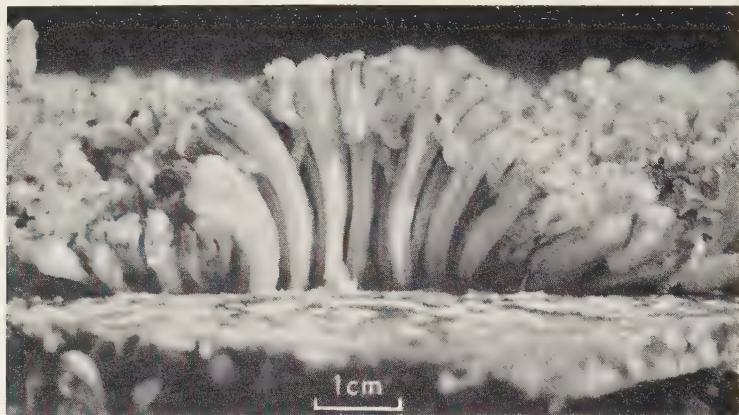
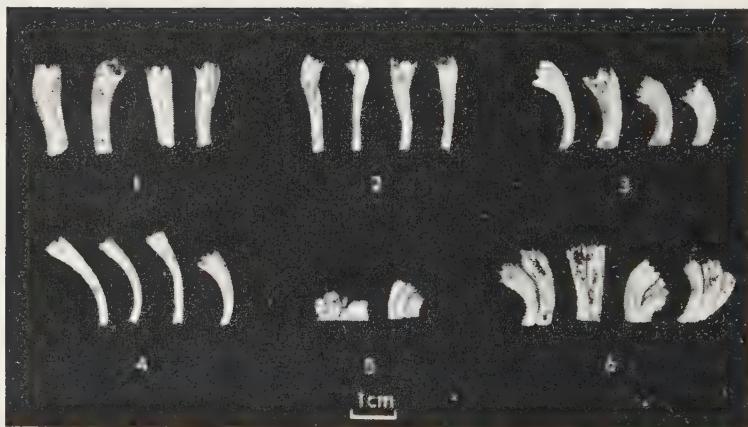


FIG. 25.1. (Upper) HUMMOCKS OF *Balanus crenatus* SHOWING THE EFFECT OF CROWDING ON THE GROWTH PATTERN OF THE INDIVIDUAL

The picture is somewhat complicated by a later spat fall of another species.



(Lower) REPRESENTATIVE INDIVIDUALS FROM VARIOUS REGIONS OF THE HUMMOCK  
(From Barnes and Powell, 1950)

also contain much that is relevant to an understanding of the growth process itself. Some of the neurological mechanisms affecting growth are subtle and very intriguing, and emphasize that the control of growth is not purely chemical, even within the body. Among inanimate external factors physical forces play an even more important part.

## 25.2. Abiotic Factors

The term "abiotic" (p. 398), though not ideal, is preferable to "inanimate" or "inorganic." The last covers only chemical substances and is meaningless when applied to physical forces. "Inanimate" has the disadvantage that logically it includes food and other materials of dead organisms, which for most purposes of growth-control should be considered along with the materials of living individuals. The only disadvantage of "abiotic" is that it also is rather meaningless when used as description of physical forces; a physical force originating from within a living organism does not necessarily differ significantly from the same force when it originates externally, and indeed the two will usually be considered together. "Biotic" and "abiotic" have the advantage that they may be taken to indicate only the source of the factor under consideration, without implications about its nature. They are preferable in the case of chemical factors also, and avoid the question whether there is any real distinction between organic materials in general and biological materials in particular. It is convenient to classify abiotic factors under the three headings: chemical, physico-chemical and physical.

### 25.2.1. Chemical Factors

No doubt extraneous chemicals do not affect growth unless they react significantly with the living systems. Metallic gold, stainless steel and plastics can be quite harmless in the body and are treated merely as mechanical factors. On the other hand probably few substances fail to react appreciably with biological materials and to affect growth. Many of them have been encountered sufficiently regularly in the past to have acquired biological significance (Needham, 1959). They cannot affect organisms unless they are absorbed or digested, when they become internal agents; the more important of these have been considered in Chapter 16. The type of substance not dealt with there is the organic compound which is not a major biological substance. The carcinogens (Table 8.1, p. 103) show what a critical effect such organic materials can have on growth. However, short of a comprehensive review, it is not easy to do justice to this very large class of substances. There is not space here for a complete review, which, moreover, might not be proportionately enlightening.

An extraneous chemical which might be singled out here is oxygen. Like food in general it is an external factor which becomes a fundamentally important internal one. Its uptake and distribution in the body can be limiting factors for growth, though in fact they are rarely allowed to become limiting. The general significance of oxygen in respiration for the support of growth has been considered (p. 260). Like other external factors it can trigger off growth responses appropriate to its utilization. In insects oxygen deficiency induces proliferation of tracheoles (p. 350), a growth response which in return improves the oxygen uptake of the tissues, a typical regulation by negative feedback. In vertebrates oxygen deficiency promotes erythrocyte proliferation (p. 95), an analogous feedback response which again nullifies the effect of the initial stimulus, and

restores the steady state. There is a limit to the concentration of oxygen which is beneficial; in *Tetrahymena* growth is retarded at tensions greater than 1·6 atmospheres of pure oxygen, about eight times normal (Phelps, 1953).

### 25.2.2. Physico-chemical Factors

Chemical agents are relatively specific in structure and action and so demand individual treatment, but physico-chemical factors are more generic and can be considered under a few main headings. These are the generic properties of ions, atoms and molecules. Only the most important will be considered here: osmotic properties have been dealt with in connexion with water as a controlling agent (p. 266) and some have been covered in considering the synthesis of macromolecules (Chapter 13). There will be scope for a more detailed and specific treatment of some of these properties when more evidence is available.

#### 25.2.2.1. Hydrogen Ion Concentration

Since protoplasm and its functioning are based on an aqueous medium (p. 266), the pH, or the ratio between the two ions  $H^+$  and  $OH^-$ , is all-important. It has been seen that the correct pH is critical for protein synthesis (p. 193), for fat synthesis (p. 239), for enzyme induction (pp. 211, 213), for the polymerization of peptide molecules (p. 170) and other growth processes. Growth is at least as sensitive as adult functions to departures from the normal value, which is around pH 7·0 for body fluids and somewhat less for the interior of cells. Foods have much the same range of pH, and rain-water has few free ions, so that terrestrial animals are rarely stressed by external acids and alkalis in quantity. Aquatic and subterranean animals, by contrast, may be subjected to the excess ions of an unlimited environment, and although the pH may not be far from normal the total work for their buffering systems can be very heavy. No doubt this is one of the major factors in conditioning an aquatic medium for growth (p. 401).

The isoelectric point of proteins is on the acid side of neutrality and they are most sensitive to damage at this point, so that in general acids inhibit growth and alkalis in moderation accelerate it. It will be recalled (p. 193) that protein synthesis is favoured by an alkaline, and proteolysis by an acid pH. In some cases a neutral pH has been found to give the most rapid growth, for instance in *Hydra* (Loomis, 1954), *Paramecium* (Darby, 1930), and cells *in vitro* (Mayer, 1939), but more usually, perhaps, a distinctly alkaline medium is best (Darby, 1930; J. Needham, 1931; Southern *et al.*, 1935; Gordon, 1957; M. E. Brown, 1957). The blood of cancer patients tends to be more alkaline than normal, and newt embryos give their most alkaline reaction when cell division is most rapid. Actively growing regions are usually alkaline to less active regions (Needham, 1952, 1960a), and in tissue cultures alkali is produced by the growth activities themselves (Mayer, l.c.), perhaps an aspect of conditioning the medium. Cell growth is maximal at a higher pH than cell division (Darby, l.c.; Richards,

1941), and this is probably a further indication (p. 261) that it requires the more fully aerobic conditions.

Aeration permits growth at a lower pH than under anaerobic conditions, presumably, by driving out CO<sub>2</sub> and through the relative alkalinity of the products of complete, as contrasted with glycolytic, respiration. For the facultative heterotrophe *Astasia longa* (Schoenborn, 1947) the optimal pH may be lower on a tryptone acetate diet than on an inorganic medium, no doubt because of the buffering power of the diet itself.

### 25.2.2.2. Electrochemical Factors

Living organisms are frequently exposed to weak electrical fields, under natural conditions, and changes in pH and in other ion concentrations produce electrochemical effects. These commonly lead to differentials across membranes, and to other potential differences which affect growth as well as other activities. Living organisms spontaneously develop their own electrochemical potentials in association with all activities, and these could have been considered at an early stage, as internal factors. However, the interaction of imposed potentials, particularly those used experimentally, with the intrinsic fields is the simplest way of studying both. Most tissues have "resting" potentials which are modified during their physiological activity, and in addition potentials arise as a result of basal metabolic processes. These also tend to vary with activity, both spatially and temporally.

The surface charge on bacteria changes with age (James, 1957), and in these simple organisms it is likely to be associated with growth, as the main activity. The charge in fact is known to be related to the intake of amino acids into the cell (p. 184). Potential differences are regularly found between growing and static regions of the body, the former usually being the more electropositive, but not always (Barth, 1934); cancer cells, for instance, have a more electronegative surface charge than normal (Purdon, 1958). No doubt much depends on the precise siting of the electrodes, though the pH optimum (*above*) would seem to imply that electronegativity should usually favour growth. Much depends on whether the observed potential is the agent for, or the product of, growth. The recovery phase following nerve conduction activity is presumably anabolic and involves the restoration of an externally positive polarization, and Hering long ago correlated anabolism with anodic polarization. Alkaline conditions might favour growth by neutralizing the positive ions responsible for the observed electropositivity of growing tissues. It would then be implied that these are an inhibitory product of growth.

A potential difference of 1 to 7 mV has been recorded between cancerous and normal tissues, and similar or larger potentials between regenerating and intact regions; the latter are at first positive and then negative to the intact region (Crane, 1950), a complication which may be relevant to normal growth also. In regenerating tissues it is correlated with a corresponding diphasic change in pH. In the growing regions of plants, potentials as high as 70 mV have been

recorded between the active tip and a point 2 cm behind it, that is 3·5 mV per mm (Lund, 1947). In an amphibian embryo a potential as high as 700 mV was measured between the most and the least active region (Burr and Lane, 1935). The resistance of living tissues is variable, but 20,000 ohms per mm is an average value (Lund, l.c.), so that under a potential of 3·5 mV per mm a current of 0·2  $\mu$ A might be flowing continuously. Potentials may fluctuate, spontaneously and rhythmically (Lund, l.c.).

Lund distinguishes three components of the whole potential, in a typical growing tissue: (1) neuroid and metastable, and therefore easily depolarized by any stimulus, but equally rapidly regenerating, (2) not easily polarized, CN-sensitive, easily maintained by continuous respiratory activity, and maintaining a steady ion flux, and (3) an obscure component, reversibly polarizable and not CN-sensitive. The first may be concerned with rapid responses to external factors as stimuli and the second with respiration, as the driving force for growth itself. After oxygen deprivation it shows a rebound increase, so that other components of the process which generates it presumably continue under anoxia, for instance non-ionic redox reactions (Marsh and Beams, 1952; Cater and Phillips, 1954). This second component is responsible for most of the observed 0·2  $\mu$ A flow of current. The third component may be due simply to the free ions of the tissue but there could be other contributory items.

One component at least of this intrinsic potential is ultimately related to the motivation of growth, since Burr (1950) found that those cotton seeds with the highest potential, ranging up to 40 mV, germinated first. Again, in the oat coleoptile tropistic bending, which is a growth process, is preceded 20 to 30 minutes earlier by an electrical potential change (Lund, 1947). Moreover, imposed potentials, which summate algebraically with the intrinsic potential, affect the growth rate, normally speeding it if they increase the intrinsic potential and vice versa (Barth, 1934; Lund, l.c.; Marsh and Beams, l.c.); clearly the intrinsic potential is largely a driving, and not a product potential. Gravity and light, which affect plant growth, cause a rapid change in intrinsic potential while X-rays, which inhibit growth in tumour cells, also cause a fall in electrical potential (Cater and Phillips, 1954).

In plants Lund found no speeding by concurrent applied fields but counter currents inhibited growth completely at 1·6  $\mu$ A. Marsh and Beams found positive concurrent effects while counter currents inhibited only above 16  $\mu$ A per mm.<sup>2</sup> The strength of current necessary to inhibit growth is three or more times that needed to polarize completely all free ions, Lund's component (3), so that growth is not related mainly to this component. However, the movement of free ions is known to play some part in growth processes, and the intrinsic potential itself is within the range capable of inducing and controlling free ion movements. Currents strong enough to inhibit growth completely are lethal if applied for a long time. Mitosis is inhibited by brief currents of high voltage (Holzbauer *et al.*, 1952), and these therefore may act on Lund's component (1), the main growth potential being insensitive to currents of this type (Lund, l.c., p. vi).

Since electrical fields are polarized, the growth associated with them tends to be orientated. It is usually maximal on the side facing the cathode of an external inducing circuit so that if this is applied transversely to a hydroid stolon or a plant root it bends towards the anode. Similarly hydranths grow out from a reconstruction mass of *Obelia* towards that side which faces the anode of the applied field (Lund, *l.c.*). By contrast hydranths of *Pennaria* and *Tubularia* regenerate best on the cathode-facing end of isolated pieces of stem; this may be because they are unable to bend. In any case tropistic bending in hydroids is a motor and not a growth response as it is in plants. The orientation of nerve fibre growth also may be sensitive to electrical fields (Marsh and Beams 1946). However, Berry *et al.* (1947) observed growth promotion in lateral roots and inhibition in the main roots of the onion, irrespective of the direction of the current.

Intrinsic potentials which can become orientated in a simple way throughout a mass of tissue could provide a possible basis for the long distance coordination of growth in animals (Moment, 1953). Organizer regions in morphogenesis are found to be polar ends of main gradients of electrical potential. Moment has developed the interesting thesis that electrical potential gradients determine not only the course but also the eventual termination of growth, for instance in annelids: their growth programme thus may be integrated in space and time by an electrochemical field, pervading the body throughout (*cf.* Becker, 1963).

A rheoscopic type of experiment, that is an attempt to stimulate growth in one tissue by the natural potential of another, should prove instructive, since it might show whether the intrinsic potential is the driving force or merely the by-product of growth. The experiment has been less popular than the attempt to demonstrate electromagnetically radiated effects (p. 422), and no doubt it would present technical problems. The general conclusion, from the facts presented above, is that a substantial part of the potential is a driving force.

Positive effects on the growth of eggs and even of plant seeds by electrostatic fields, in air, were detected by Romanoff (1935). Growth was speeded by the brush discharge from a cathode 35 cm away. This is relevant to the possible effect of natural ionizations by lightning, auroral discharge, and so on. Many, though not all, early experiments showed positive effects (Davenport, 1899). In most cases growth was accelerated. Growth was not polarized, however, as it should have been, and secondary effects were suspected. Currents through the soil have also been found to promote growth, and these certainly might have caused heating as the major factor. Inhibition was obtained in a careful recent experiment on fungi (Rosen, 1961). Electric fields in air affect the behaviour of Hymenoptera (Husing *et al.*, 1961) so that there is no reason to doubt their ability to affect living processes.

### 25.2.3. Physical Factors

Chemicals can act only in contact, at least by macroscopic standards (Rothen, 1956), but physical agents, depending on the movements of subatomic

particles, travel rapidly and penetrate matter more freely. They are effective from very distant sources, the sun in particular. Physical factors nevertheless become incorporated into living systems, in such forms as heat and light energy; autotrophes convert light into chemical energy, stored as chemical compounds, which thenceforth are internal factors.

By virtue of their pervasiveness, speed, power and other properties, physical factors are particularly fitted to serve purely as stimuli, or triggering agents, for active growth responses by the organism, and this type of response must be distinguished carefully from the direct effect of the same agent. For instance the direct effect of an increase in temperature is to speed chemical reactions and most other processes, but as a triggering agent it may induce a great variety of responses; these are biological responses as opposed to passive physical effects. There is a third type of response to external factors, and this is not necessarily more evident for physical than for chemical agents, namely the ability to antagonize or neutralize the direct effect, when this is deleterious. Living organisms survive only because they have this ability. Thus cold slows down growth and other activities; yet fish in the North Sea grow virtually as rapidly as individuals of the same species, living at a temperature 10° higher, in the Mediterranean (Heilbrunn, 1952). This is a complex response of the whole organism and another essentially biological type of response, a manifestation of physiological homoeostasis.

#### **25.2.3.1. Magnetic Fields**

Early work (Davenport, 1899) indicated that electromagnetic activity might influence growth, and there has been recent evidence for this from more refined techniques (Audus, 1960). The remarkable superficial resemblance between the division spindle of the cell (Fig. 10.3) and the lines of force around a magnetic dipole, as revealed by iron filings, proved to be purely analogous, and this tended to discredit the more general possibility that magnetic phenomena could have anything to do with growth, or indeed with any activity of living material (Heilbrunn, 1952, p. 569). More pertinently no effect on cell division by extrinsic fields as powerful as 35,000 gauss could be detected by Milovidov (1954), and others could find no effect on the growth of hens, trout and silkworms (J. Needham, 1931, p. 537). However, powerful electromagnetic fields have now been found to cause chromosome abnormalities in dividing cells (Teixeira-Pinto *et al.*, 1960), which perhaps reopens the classical question. Mulay and Mulay (1961) find 4000 gauss enough to destroy ascites cells.

It was once suggested that the preponderance of the one isomer of optically active molecules, in living materials, might be determined by the asymmetry of the earth's magnetic field, but there is strong evidence against this. One argument is that if the earth's field had this power it should also be capable of orientating plant growth, which in fact is typically symmetrical. The orientating effect on root growth (Audus, *l.c.*) demands stronger forces than the earth's field. Orientation is likely to be the most important effect, and it is interesting

that F. A. Brown *et al.* (1960), find that snails orientate their locomotion in a magnetic field. Strong electromagnetic fields orientate the movement of protozoa and their cell inclusions (Teixeira-Pinto *et al.*, 1960). Arnold (1958) has suggested that muscle fibres may be orientated by their own diamagnetic properties; on drying, some muscle fibres become paramagnetic along, and diamagnetic across, the fibre axis. Rothen (1952) found that magnetic fields orientated protein molecules deposited as monolayers from solution; moreover the resulting protein layer was able to react with enzymes and antibodies not in immediate contact. This seems to support the views of others that chemical activity can occur as a result of electron conduction induced over considerable distances by magnetic resonance in a hydrogen-bonded continuum of proteins and water (Szent-Gyorgyi, 1961). However, this has been strongly criticized (p. 204).

The belief that proteins might be synthesized by their intrinsic resonance forces has been considered adversely (p. 204) but there appears to be considerable evidence, nevertheless, that magnetic phenomena do have some significance in vital activities. Semiquinones and other organic substances with a number of double bonds in the molecule are weakly paramagnetic and some organic materials are diamagnetic, repelling the lines of magnetic force. Each  $-\text{CH}_2-$  group of a carbon compound in solution contributes to the rotation, by an imposed magnetic field, of a beam of light passed through it. Light induces paramagnetism in chloroplasts (Commoner *et al.*, 1956), and may help in liberating the active free radicals essential for photosynthesis (Calvin, 1962). The presence of iron in haemoglobins, cytochromes and other enzymes is a strong *a priori* reason for expecting measurable magnetic phenomena in normal metabolism. Haemoglobin changes from para- to diamagnetic when it becomes oxygenated (Keilin and Hartree, 1949). Some similar significance may be expected for the presence of cobalt (p. 274) in vitamin B<sub>12</sub> (p. 316) and for the carcinogenic properties of cobalt (Table 8.1).

In fact, recent work in general has shown a significant effect of magnetic fields on growth. Barnóthy and Forró (1948) found that  $3-6 \times 10^3$  oersteds caused temporary retardation of the growth of female mice and inhibited cell division. This field strength also inhibited tumour growth (Glaser, 1960). Mulay and Mulay (1961) found that a field of 4000 gauss from a permanent magnet was sufficient to inhibit the growth of mouse ascites cells and even to kill them. Tumour cells have a magnetic susceptibility different from normal (Senftle and Thorpe, 1961). This may be a reflection of their high iron content (p. 99).

#### 25.2.3.2. Pressure

Animals live and grow in the Tonga ocean deeps, at 35,000 feet, as recent dredging expeditions have shown, and saltacid spiders have been found established at 22,000 feet on Everest, so that growth must be possible up to 1200 atmospheres and down to one-third of an atmosphere. Experiments with turkeys

previously accustomed to normal atmospheric pressures indicate that their growth is not appreciably affected at a pressure simulating an altitude of 10,500 feet (Smith *et al.*, 1954). There is a small effect on rodents in the second generation at 15,000 feet (Moore and Price, 1948), and subnormal lactation by the first generation. At 25,000 feet there is considerable retardation of growth in the rat, even in the first generation, particularly in the male (Altland, 1949). At this height there is a faulty deposition of dentine, but not of the organic matrix of the teeth (Gersh and Restarski, 1944). Older records indicated that the growth of plants may be accelerated at moderately low pressures (Davenport, 1899, p. 368) and also at mildly supernormal pressures.

The effect of high pressures has been reviewed by Johnson *et al.* (1954). Deep-sea bacteria grow better at 400 to 600 atm. than at sea level but organisms accustomed to 1 atm. all show retardation, which may be detectable at 68 atm., and is virtually maximal at 300 atm. in *Escherichia coli*. Cells *in vitro* are said to grow and proliferate at pressures up to 1850 atm. (Benthaus, 1937, 1941), which is greater than that of the deepest ocean. The cells therefore are less sensitive than the growth of the whole animal from which they came: growth as a whole is more sensitive than particular components. This is shown again by the observation that enzyme activity is possible up to 1000 atm.; pressure actually protects some proteins from denaturation. Cell division seems in general to be more sensitive than this, notwithstanding Benthaus's results: *Ascaris* eggs may divide up to 800 atm., but most cells not beyond 200 to 400 atm. The division furrow is prevented from developing and one already started recedes. Viscosity is one of the critical properties affected; amoeboid and muscular movements cease at around the same value, 400 to 430 atm. Bacteria continue to grow without cell division, and produce the familiar mycelial forms induced by so many abnormal conditions. A pressure of 600 atm. retards the multiplication of bacteriophage viruses but clearly synthesis processes are less sensitive than cell division. In developing eggs an effect on the state of DNA has been suspected and this is not improbable in view of its large molecular size.

At worst, the pressures tolerated are surprisingly great, and contrast with the small latitude tolerated in osmotic pressure (p. 266). No doubt much depends on the pressure being uniform throughout, and very small asymmetries are disastrous. Small differential hydrostatic pressures may stimulate growth, however, and are often utilized by animals in particular situations, as in the growth of nerve fibres (Young, 1945a), the growth of the stolons of hydroids, and the postecdysal size increase of aquatic arthropods (p. 20). The sea anemone, *Cerianthus*, cannot regenerate from small pieces if these are not permitted to develop a positive hydrostatic pressure (Hyman, 1940, p. 632). In hydroids pressure in the entocodon is maintained by peristaltic contractions in the coenosarc (Huxley and De Beer, 1923; Hauschka, 1944; Berrill, 1949a). Removal of the perisarc allows distension of the tissues locally and so increases growth there. A similar distension of the body of the bug, *Rhodnius*, by the large meal of

blood it consumes, appears to be the effective stimulus for growth (Wigglesworth, 1945); the full effect is developed only in the next instar. Plant cell growth depends very much on internal hydrostatic pressure.

High pressures can protect against high temperatures, possibly simply on account of the gas law,  $PV = RT$ ; high pressure may prevent the expansion due to increased temperature. Under high pressure some bacteria can grow at temperatures which would be completely inhibitory at atmospheric pressure.

Differential mechanical forces play a significant part in growth control. Bones strengthen along lines of pressure and tension (p. 77). Pressure from the inside causes resorption on the inner surface of the cranial bones (p. 79). Fluctuating forces tend to cause strengthening, and steady forces resorption.

#### **25.2.3.3. Ultrasonic Vibrations**

Mild shaking stimulates the growth of bacteria but more vigorous treatment is inhibitory, and fatal if prolonged (Davenport, 1899, p. 371). There is some disorganization of protoplasmic structure and this becomes increasingly serious at higher frequencies of vibration, in the upper audible and ultrasonic ranges, provided the intensity also is adequate. At low dosage ultrasonic agitation stimulates growth (Graber, 1953), possibly for the same reason as for many other destructive agents, namely that regenerative processes are induced at a more rapid rate than the destruction. Microorganisms are most sensitive to the deleterious effects of vibration in the logarithmic phase of the culture, when proliferating most rapidly. Tumour cells are more sensitive than normal cells, and ultrasonic treatment has been used to destroy them differentially.

The treatment causes denaturation of proteins and inhibition of enzymes, aberrations of both nuclear and spindle apparatus during division, and cavitation in the cytoplasm, that is vacuole formation due to the phase of negative pressure in the vibrations. Free oxidizing radicals are released, as by short-wave irradiation (p. 421), and oxidative metabolism therefore is accelerated. In moderation this may promote growth. The heating action of the agitation is not the critical factor inhibiting growth at higher dosage.

#### **25.2.3.4. Temperature Change**

Apart from radio waves, heat or infra-red waves are the longest of the electromagnetic spectrum and constitute a large fraction of the total solar energy reaching the earth. For living organisms heat is the most important external physical agent, other than light, and in its direct effect on the growth of animals it is far the more important of the two. Light promotes some reactions but heat speeds all. Its importance is indicated by the acquisition of homoiothermy by mammals and birds, a means of keeping body temperature near the upper end of the terrestrial range. The constancy of the body temperature in these groups is of course a second important consideration.

The direct effect of an increase in temperature is to speed growth as it does most physiological processes, chemical and physical. If growth rate is plotted

against temperature a sigmoid curve is usually obtained (Wigglesworth, 1953), which differs from that for inorganic reactions in its inflection; this is due largely to the inactivation of proteins, through denaturation, towards the upper end of the physiological range. The exponential relation of van't Hoff,  $v = a \cdot k^T$ , therefore does not apply; the ratio of the rates at two temperatures  $10^\circ$  apart, the  $Q_{10}$ , is not constant but declines continuously with increase in temperature. Some growth curves have been fitted with some success by modifying the above relation to include a term for the rate of protein denaturation, which itself is thought to obey a van't Hoff relation. The complete expression therefore is of the form—

$$v = 2v_m(k^{T-T_m} + k^{T_m-T})$$

where  $v_m$  is the maximal rate and occurs at temperature  $T_m$ . This relation is of the same general form as the logistic (p. 13) which in fact has been fitted to some curves of growth rate/temperature—

$$v = k/(1 + e^{a-bT}).$$

By analogy with the logistic relation for size/time (p. 14) the acceleration with increase in temperature,  $dv/dT$ , should be related to velocity,  $v$ , itself by a relation of the form—

$$dv/dT = (b/k) v (k - v)$$

This could imply that the acceleration of growth due to temperature increase is the product of two factors, one increasing and the other decreasing with  $v$  itself, a positive and a negative feedback term.

The orthodox logistic relation would give a velocity which tends asymptotically to a limiting maximum, but in fact growth and other processes pass through a maximal rate at a temperature short of the lethal point, and the rate then progressively declines. Short of the irreversible lethal range the retardation is reversible by simply lowering the temperature. This part of the velocity/temperature curve is recognized by the modified van't Hoff relation above, and the logistic likewise can be modified to cover it (Wigglesworth, 1953).

Considerable use has been made of the Arrhenius relation,  $v = ak^{1/T}$ , which is not so incompatible with that of van't Hoff, over the physiological range of the absolute scale, as its form might imply, since at these high absolute temperatures  $T$  is almost linearly related to  $1/T$ . In inorganic reactions the Arrhenius relation often fits experimental results much better than that of van't Hoff, but for data on growth the improvement is small compared with the marked deviation from either, over the upper part of the physiological range (Wigglesworth, l.c.). Arrhenius's relation is used in the form—

$$v = v_0 \cdot e^{\mu/2(1/T_0 - 1/T)}$$

the constant  $\mu$  being called the thermal increment or temperature characteristic. It has been held that  $\mu$  does not decline continuously with increase in temperature

in the same way as van't Hoff's temperature coefficient,  $Q_{10}$ , but that it is constant over considerable ranges of temperature, changing abruptly at one or two critical points. Some tests of the validity of this contention are that the position of the critical points should correspond in most physiological processes within one organism, or between, say, the growth responses of most organisms, and that the actual values of  $\mu$  should be comparable under these same conditions. The results have not stood this test, and moreover the fitting of actual results in this way is very much a subjective matter: some sets of results seem to imply that  $\mu$  is as continuously variable as the  $Q_{10}$ .

As an approximation, a simple linear relation will fit the main, central part of a sigmoid curve, and entomologists have made much practical use of this. Another which merits consideration for its empirical ability to fit actual results is the power relation of Belehradek (1930),  $v = aT^k$ , the logarithm of the velocity being linear in temperature. It includes the linear relation as a special case,  $k = 1$ . As yet no generally accepted theoretical basis has been found for it, however, and without modification it implies no limiting velocity, and so is less plausible than the modified van't Hoff relation.

Probably growth is too complex to be defined adequately by any of these relations, as in the case of the size/time relation (p. 14). Nevertheless relations such as those of van't Hoff and Arrhenius are valuable for comparative purposes, just as in the case of the allometry relation (p. 35), and particularly in view of their theoretical basis. Physical processes have a  $Q_{10}$  of 1·0 or little more, but chemical reactions one of 2·0 or more. Biological processes, including growth, prove to have a value of 2·0 at a point near the middle of the physiological range, indicating that chemical rather than physical processes set the pace.

It is possible to supercool living organisms (Billingham, 1955; Smith, 1958) to indefinitely low temperatures, short of absolute zero, and growth in some continues below 0°C (Scholander *et al.*, 1953; Heilbrunn, 1952). However, it tends to zero rate within 10 to 20 degrees of the freezing point of water, and this is predicted also by the steep increase in  $Q_{10}$  with decrease in temperature. Both this and the falling  $Q_{10}$  at high physiological temperatures are probably biological adaptations, bringing activity to a standstill at temperatures where ice formation normally would make them impracticable in any case, while making activity much less temperature-dependent in the important, upper physiological range than it would otherwise be. The temperature at which activity ceases is sometimes called the physiological zero. Tissues stored at temperatures below this, with precautions against freezing and other damage, retain, at least for some months, the ability to resume growth at normal temperatures; the time is greatly extended by the use of glycerol as an antifreeze and an osmotic agent.

The temperature which gives the maximal growth rate is usually called the optimal temperature, but this depends on a somewhat dubious ethical judgment about maximal rates (p. 279) and perhaps the less committal "maximal growth temperature" (m.g.t.) would be preferable. The m.g.t. varies in different animals. In tropical and temperate latitudes it is higher in terrestrial than in

fresh-water animals, and higher in these than in marine types; in fact it is related to the normal environmental range. Growth rate itself is similarly adapted (Heilbrunn, 1952) so that the rates of all can be very similar at corresponding points in their environmental temperature ranges. As already noted, individuals in a cold sea grow at about the same rate as individuals of the same species living in a normal medium ten degrees warmer, and considerably faster than the latter when first transferred to a common temperature. It seems probable that the underlying mechanism is related to that causing the anomalous  $Q_{10}$  property. A stock from one environment quite rapidly readjusts when transferred to the other, so that the thermostat mechanism is not rigidly fixed. Its adjustability is a typical example of the third type of response (p. 412), tending to counteract the direct effect of environmental change. The tissues of homoiotherms have become adapted to grow well only within a narrow range around normal body temperature, 37 to 40°C in the chick. Spermatogenesis probably retains an atavistic preference for a lower temperature, which may explain the descent of the testes, in some mammals, into an external scrotum, and their location near the air sacs in birds (Cowles and Nordstrom, 1946). In the sparrow spermatogenesis is most rapid at night when body temperature is lowest—but also when the demands of other activities are minimal (p. 18); experimental work shows that a high temperature does actually inhibit this productive activity. Nevertheless the testes of many birds and mammals are inactive in winter, for biological, adaptive reasons. While marine organisms are very intolerant of high temperatures, thermophilic bacteria live and grow at temperatures up to 70°C (Hinshelwood, 1946, p. 254). Fox (1957) envisages that this may be nearer the pristine environmental temperatures than normal, present-day temperatures; at any rate there is extensive adaptability to different temperatures. Environmental temperatures can vary greatly with season, particularly on land, and even the diurnal cycle may have a large range. Perhaps adaptation to this explains why the growth of the fish, *Cyprinotus*, is more rapid on a diurnal rhythm of temperature than when kept uniformly at the highest temperature of the day (Klugh, 1927). Some plants likewise grow best on a diurnal cycle of temperature, and may even demand it (Went, 1954).

In young trout, Margaret Brown (1946) found two temperatures of maximal growth, at 7 to 9°C and 16 to 19°C, giving a bimodal curve. The explanation suggested is that locomotor and other activities are maximal at a temperature half-way between these two, and that their competition for fuel and material therefore depresses growth rate most in that range. This is perhaps a good demonstration that some animals operate on a very narrow margin of spare materials and energy, a situation which does not normally apply to children because of their leisurely growth (p. 257). However Brown's fish were fed *ad libitum* so that the competition may lie in the machinery rather than in food and fuel. Her results are also a warning that a recorded maximal growth temperature may not be purely the characteristic of this one process but rather of its differential response within the totality of physiological activities: all of these

should be considered. At hatching the embryo of a poikilotherm growing on a fixed supply of yolk is smaller at a high than at a low temperature (Gray, 1929a; Uvarov, 1931; von Bertalanffy, 1949) because maintenance activities (p. 253) are differentially accelerated, and make a relatively greater demand; in this case, it will be noted, the efficiency of growth is decreased.

In homoiotherms the efficiency of metabolism is maximal not at a low, but at body, temperature so that optimal and maximal growth rates are virtually identical. At lower temperatures much energy which a poikilotherm could use for growth is used to keep the homoiotherm warm. This is such a large item that farmers have been amazed how little food cattle need for maintenance in a sunny summer (Easterbrook, 1959); the calves, however, do feel the strain of the shortage of grass in such a season, because they need a substantial margin for growth. Homoiotherms tend to be relatively large in high latitudes (Bergmann's rule) since their heat loss is proportional mainly to surface area, which is relatively smaller in large animals. Poikilotherms show the opposite tendency, to be small in high latitudes since their critical need is to absorb as much heat as possible when available.

The components of the growth process are differentially affected by temperature change. Fish produce the maximal number of vertebrae and other meristic parts at various temperatures, each different from that of maximal body growth (Tåning, 1952; Gordon, 1957). Further, the vertebral number and other individual components have a different temperature curve in different species, such as *Salmo* and *Fundulus*. Homoiotherms produce shorter extremities if they grow up at a low temperature (Harrison *et al.*, 1959). This reduces surface area and heat loss. There is also a genetic predisposition for homoiotherms living in high latitudes to have relatively short appendages (Allen's rule), irrespective of their personal life history. In insects a high temperature causes a relatively low production of the juvenile hormone (p. 381) and so the insect metamorphoses and matures at an earlier growth stage (prothetely), while at low temperatures there is the converse phenomenon (Comfort, 1956, p. 147). As already noted (p. 114), cell division in insects reaches its maximum at a lower temperature, 20°C, than cell growth (Bodenstein, 1953); above that temperature, therefore, mean cell size increases. In many cases growth continues at temperatures which halt cell division completely: once more (p. 414) the latter is the more sensitive. Bacteria and schizomycete fungi give mycelial forms in consequence. Intermittent high temperatures have been used to suppress cell division differentially and so bring all members of a protozoan culture into the same pre-mitotic stage (p. 341). It is probably significant that DNA synthesis has a temperature coefficient twice that of cell-division (Maaloe and Lark, 1954). In hydroids, however, Crowell (1957) found that cell proliferation was accelerated up to maximal temperatures consistent with survival, but that general growth was maximal in medium to cool conditions. Cell division seems to be the more easily inhibitable by cold, also (Spear, 1928).

Pressure may affect the response to temperature (p. 415) and some other

factors also modify it. An increase in food intake may lower the m.g.t. (Schoenborn, 1947), perhaps by its "specific dynamic" release of energy (p. 254). An increased oxygen supply may raise the upper limiting temperature, for regenerative growth in marine animals, by as much as 5°C (Barth, 1955).

As a triggering stimulus temperature is often the factor which breaks diapause in insects, while cold is commonly the factor which induces its onset. A rise in temperature is sometimes the stimulus to animals to begin seasonal breeding activity but in these functions, as in growth, it is less common as the triggering agent than light. The amounts of the two in solar radiation are correlated but light is the more consistent as a seasonal signal. Another response to cold, which is also a good example of a counteracting response, is the increased growth of fur by mammals in winter (p. 17).

#### 25.2.3.5. Light

Indirectly light is the source of energy and food for animals. Its direct significance for animal growth is more limited, to such cases as the promotion of vitamin D synthesis in mammals and birds. The beneficial effect of light on the growth even of such well-furred mammals as the rat (Luce-Clausen and Brown, 1939) and cat (Davenport, 1899) may be due to this factor. Another direct beneficial effect is to neutralize the inhibitory action of rays of shorter wavelength, particularly of the ultra-violet. There are other possibilities in some cases; tadpoles and the snail, *Limnaea*, grow better in the light (Davenport, l.c., p. 422) and this may be due to the production of algal food. However, Eisler (1957) found that darkness reduced the growth of salmon fingerlings even on a reasonably balanced and complete diet, such as liver. The fish *Cyprinotus* grows progressively more slowly as the light intensity is reduced (Klugh, 1927) and *Astyanx* also requires light (M. E. Brown, 1957). It seems possible that the slowing of growth in winter may be due partly to light restriction.

For the growth of some animals light is not important and it actually inhibits that of *Mytilus* (Huntsman, 1921), *Balanus* (Costlow and Bookhout, 1956) and the chick embryo (Davenport, l.c.). At the cellular level its effect seems to be more consistently inhibitory: for instance the growth of carcinoma is arrested (Morton *et al.*, 1940). It appears to have a direct effect on the division spindle and causes one already forming to regress (Datta, 1960). With the enhancing effect of a photosensitizing dye light causes a cessation of growth, and of the synthesis of protein and DNA (Hill *et al.*, 1960). Some instances of increased growth rate at night are due to the beneficial effect of rest (p. 18), not to the absence of light, and in nocturnal animals growth is maximal during the daytime. Light is generally inhibitory to the growth of plants, an apparent paradox which, nevertheless, is biologically useful in orientating growth: shoots grow faster on the side away from the light and therefore bend towards it, to the benefit of photosynthesis. In darkness rapid growth, combined with this phototropism enables shoots to reach any available outlet into the light. Hydroids and other sedentary animals usually grow with a definite orientation,

either directly towards or away from light. As already noted (p. 411), this is not due to differential growth of the type seen in plants, but in hydroids to a primitive contractility similar to that which causes hydrostatic changes (p. 414). There is also a tendency, in some animals, to put out buds mainly on the relevant side, a true growth response. Light inhibits the cell division spindle (Datta, 1960).

Light is known to be a triggering stimulus for growth in a number of animals and this is suspected for others. It induces the breaking of diapause in some insects (Way *et al.*, 1949; Andrewartha, 1952; Lees, 1955); the diversity of factors which can break diapause indicates that all are acting non-specifically as triggering agents (p. 425). Often the stimulus is a particular photoperiodicity, or pattern of light and dark periods, the pattern which normally occurs in the optimal season for growth. In some cases the length of the dark interval is more critical than that of the light (Wareing, 1953), possibly depending on whether the relevant season is one of shortening or of lengthening days. The initiation of plant growth in general is more sensitive to photoperiod than that of animals. Light acts as an inhibitory triggering stimulus when it causes the release of the sinus gland hormone in the Crustacea (p. 379), and so inhibits moulting (Bliss, 1954).

In the lizard, *Anolis*, Fox and Dessauer (1957) discovered something of the way in which light acts as a triggering stimulus. Exposure to an experimentally lengthened day of 18 hours caused an increased appetite, and so increased intake and gain in weight. The weight increase appeared to be greater than the increased food consumption so that a slight direct effect of the light on growth was suspected, presumably increasing the efficiency of utilization of the material.

Even moonlight may trigger off growth processes, such as proliferation in the ciliate *Stylonychia* (Gray, 1951), and other organisms (Seifriz, 1936, p. 406). It causes growth to have a lunar periodicity in some of the Crustacea (Wheeler and Brown, 1936). Reproductive activities, which involve growth and productive synthesis, very commonly show lunar periodicity (Fox, 1924, 1928). As a direct agent moonlight may be adequate to promote photosynthesis (Semmens, 1947). There is some evidence that the bioluminescence of bacteria can promote growth in other organisms (Harvey, 1952, p. 90), for instance cell proliferation in bone marrow and the germination of seeds.

#### 25.2.3.6. Radiations of Shorter Wave-length

In low dosage ultra-violet, X-rays and the  $\gamma$ -rays from radioactive materials stimulate growth, but this is probably through the release of regeneration-promoting substances from damaged cells (Loofbourow, 1948), and the direct effect is strongly inhibitory. Irradiation consequently has been one of the most effective agents for destroying neoplastic growth. The "hardest" rays, that is to say those of shortest wave-length, are the most effective. There is a large literature on this, including the works of Ellis and Wells (1941), Lea (1955), Spear (1953) and Hollaender (1954).

As already indicated (p. 109), the process most sensitive to ionizing rays is the synthesis of DNA (Cohen, 1959; Tobias, 1959). Irradiated rats synthesize only half as much purines as normal (Hevesy, 1949a); the decrease is mainly due to the effect on DNA synthesis, that of RNA being much less sensitive (Cohen, l.c.). Protein synthesis also is much more insensitive. DNA is much less sensitive to irradiation once it is formed than during synthesis. The action appears to be primarily oxidative (Scholes *et al.*, 1949; Collinson *et al.*, 1950; Wood, 1959), beginning in the ionization of water and the production of hydrogen peroxide, which itself inhibits growth (Sugiura and Holman, 1957; Hankin, 1958). The action on DNA makes the chromosomes sticky and this helps to make cell division abnormal.

Effects which may be independent of this but are also important for growth include the coagulation of proteins by ultra-violet light (Ellis and Wells, l.c., p. 670) and this may be the reason why spindle formation is inhibited (Tobias, l.c.). A number of enzymes are inhibited (Anderson, 1947; Pollard, 1959). There is a minority view that protein synthesis may be more sensitive to irradiation than DNA synthesis (Billen, 1959) or that nucleoprotein is the critically sensitive state of the material (Carlson and Hollaender, 1948). The syntheses of NA and protein are so closely interdependent (p. 230) that this question is not easily settled. Lecithin also is said to be inactivated (Ellis and Wells, l.c., p. 669). Irradiation of the whole body affects particularly the bone marrow and other rapidly proliferating tissues. Damage to the pituitary secondarily inhibits the growth of the whole body (Tobias, l.c.), as might be anticipated.

Visible light has considerable protective power against shorter-wave radiations (p. 420), but photodynamic substances, which sensitize other materials to the action of visible light also tend to increase the sensitivity to shorter rays (Matoltsy and Fábián, 1946). Many carcinogens are photodynamic (p. 106).

Ionizing radiations possibly never act as triggering stimuli for growth; they induce mutations in nuclei but their direct damaging effect is generally thought adequate to explain this. Apart from ultra-violet and a small amount of cosmic radiation these rays are not common external factors so that there has been no selection in favour of their use as triggering agents. It may be for the same reason that no real defence against them has been evolved by living organisms, again except in the case of ultra-violet of the wave-length band common in sunlight. The protection of human beings by sun tanning is an interesting example of induced synthesis. Cosmic radiation may have been abundant on earth at certain times (Krasovskii and Shklovskii, 1959) but there is no certain evidence (George *et al.*, 1949; Ong, 1949) that present levels affect growth.

Gurwitsch (1932) believed that he could detect stimulation of cell division, across an air gap, by short-wave rays actually produced by other proliferating cells. These he called mitogenetic rays. Some further support for the production and stimulating action of the rays was obtained (Rahn, 1934), but at best the emission was barely distinguishable above the background noise of

instruments sensitive enough to detect it. Effective stimulation within a tissue seems more possible, but unimportant compared with chemical mitogens (p. 345).

### 25.3. Conclusions

The majority of external factors are inimical to living systems because these survive only by maintaining a difference in energy potential between themselves and their environment. The potential enables them to "do work on the environment" but reciprocally the latter tends to discharge the potential, according to the laws of entropy. Living organisms must react to the external factor so as to counteract this direct effect quantitatively; within the normal range of intensity of the external factor the response is a "physiological" range of internal adjustment.

It is evident that at least one external factor must be propitious, and for animals in fact there are two: heat and food, including water and oxygen. Because of the need to perfect internal homoeostatic mechanisms against inimical external factors it is only possible for the living system to be well adjusted to a limited normal range, even of beneficial factors; extremes of temperature are therefore as deleterious as any of the environmental factors and the organism must evolve counteracting responses to deal with them.

An apparent paradox arises from this counteracting type of response. For instance some insects grow much more rapidly on a restricted intake than on an ample diet, probably for the biological reason that early maturity and reproduction are necessary if food is scarce: eggs are better able to tide over the subsequent period of famine than active insects. Carcinogens directly inhibit growth (p. 107) and neoplasms perhaps arise as an excessive reaction against this: cancer is most common in elderly individuals whose growth has long been held in check by the normal systemic inhibitor (p. 428).

Another type of paradox results from the fact that since living organisms are adapted to a particular range of each relevant external factor the deleterious effect of an excess may appear very similar to that due to a deficit: growth rate is maximal at an intermediate temperature and falls off on either side of this. Again, both overfeeding and underfeeding may cause premature ageing (p. 444). In most cases it is unlikely that the direct effect of both excess and deficit could be similar and the paradox lies rather in the mechanism of this type of indirect response by the organism.

A third type of paradox results from the third type of response to external agents, the triggered response. In one animal heat, and in another cold, may trigger off growth; in one the lengthening, and in another the shortening, day may initiate a growth cycle. With all these possibilities of paradoxical manifestations it is not surprising that the analysis of growth control presents some difficulties at this level. Further complications are introduced by the fact that an agent may have a very clear direct effect as well as triggering off an indirect response of a very different type, which need have no direct physical or chemical

connexion with the nature of the agent itself. It is a causal relationship of obscure intermediation. In most cases the mechanism of intermediation in the counteracting type of response is still equally obscure.

Some animals are protected from direct exposure to certain external factors but most of these play a significant part in the normal control of growth. The difficulties in defining this, owing to the paradoxical manifestations recognized above, are to some extent offset by the fact that external factors usually act at the highest level, on the organism as a whole. Their effect therefore is comprehensive, striking and often clear cut, comparing in magnitude with that of systemic internal factors. The subject of the next chapter is the integration of these internal and external controls at the highest level. For this purpose the most important of external agents prove to be those physical factors which act as triggering agents.

## CHAPTER 26

### *Control at the Highest Level*

THE main questions concerning the ultimate integration of growth are: Is there a single master control at the organismal level? Does this operate by the progressive deputizing of control down through systemic, organ, cellular and molecular levels? Is the master control merely modified by external agents or are these a more indispensable part of it? The first two extend the enquiry of earlier chapters and the third relates this to the findings of the previous chapter. The genetical analysis (Chapter 24) gave a picture of individual genes controlling growth at one of the various organizational levels, in quite typical Mendelian manner, and the present interest is the interaction between genes so as to deploy control down through the hierarchy of levels.

The study of external factors has shown that they frequently trigger off active growth responses in much the same way as more overt types of behaviour are evoked, through a special interpreting and co-ordinating system. This strongly implies that there is a similar integral or master control of growth which is triggered as an entity and *must* operate by deputization through the hierarchy of levels. It also indicates that the state of this master control in many non-growing bodies is a metastable one, with its resting potential very sensitive to being fired off into active growth. The state of no growth is rarely one in which the power to grow has been exhausted, but rather one of positive suspension. This is evident from the behaviour of microorganisms in culture, and of metazoan cells *in vitro*, or when they become cancerous. In cases where the resumption of growth is clearly adapted to being triggered off, the response tends to be of the all-or-none type and the inducing agent therefore appears much more potent than those which merely affect the rate of growth.

Such bodies as eggs and spores are in a typical metastable condition and need to be triggered (Gray, 1931; Heilbrunn, 1952). It is typical of triggered responses that when tested experimentally they show little or no specificity to a particular triggering agent, though often there is only one which is normally in a position to act. The entrance of the sperm is the sole normal triggering agent for development in metazoan eggs; nevertheless for some eggs mere mechanical puncture by a needle, particularly if contaminated with serum or other materials which would normally accompany the sperm, is an effective substitute for the normal stimulus. Other experimentally effective triggering agents include various chemicals, surface-active agents, and physical forces such as electric shocks and rapid temperature change, many of them quite unrelated, apparently, to the natural signal. Normally there are safeguards, including a

specially low threshold, which ensure a response specifically to the latter. Fertilization of the egg in fact involves processes with much resemblance to immunological reactions (Tyler, 1948), which are among the most specific of biological responses.

Penetration of the egg by a spermatozoon in some cases is known to set off a rapidly spreading cortical change over the entire egg (Rothschild, 1956), and this may initiate the developmental sequence itself, beginning with cell division. There is an equally abrupt increase in oxygen consumption (Barth and Barth, 1954; Boell, 1955) and a change in respiratory pathways (J. Needham, 1942), similar to that when diapause is broken (*see below*). Thus control is immediately deployed throughout the hierarchy, down to the molecular level. The cortical changes resemble in certain essentials the typical work functions, such as those of muscle and other effector organs (Brachet, 1950, p. 137), and therefore may be included, along with cell division, among the work functions associated with growth. Among their resemblances to effector functions is this same sensitivity to a wide variety of experimental stimuli, in spite of the stereotyped character of the normal triggering agent.

The phenomenon of diapause, particularly characteristic of insect development, is a more dramatic illustration for the present purpose since it can, and does, occur within the course of growth; both arrest and resumption of growth are usually very abrupt. Both are typical triggered responses, ideal for study in this context. The usual non-specificity of the triggering is illustrated not only by the variety even of their natural stimuli, in different species, but by the fact that some which induce diapause in one induce its breakage in another, depending on the particular environmental situation for which diapause has been evolved. Lees (1955) has pointed out that the normal stimulus inducing diapause is usually one which acts in advance of those adverse conditions against which the growth suspension is a necessary provision, and this again shows the essentially indirect and triggered nature of the response. Experimentally a variety of acute stimuli are effective in breaking diapause (Andrewartha, 1952). These include mechanical and electrical forces (Varley and Butler, 1933), again revealing a broad similarity to nerve and muscle stimulation. In any case the most common natural stimuli, changes in temperature or in photoperiodicity, act via the nervous system.

During diapause there is a very low output of the GD hormone (p. 381) and the cytochrome system of terminal oxidation is usually inactive. When diapause is broken this system is rapidly reactivated and GDH output suddenly increases. It seems that there is much the same kind of development of growth control, through the hierarchy of levels, as at fertilization. The external triggering agent acts via the sense organs on the highest centres of the nervous system, initiating the response at the organismal level, therefore. The brain, through its neurosecretions, evokes the activation of the prothoracic gland to secrete GDH. This hormone, systemically distributed, promotes cell division and probably activates the cytochrome system. These last two actions may be

in parallel and not in series, but at any rate key processes are operated at each level and the crispness of the response no doubt owes much to this. If a diapausing pupa is injured ecdysone is probably produced locally and activates the cytochrome system in connexion with repair, but without breaking diapause (Shappirio, 1960)!

The eggs of parasites and other specialized animals often show a type of diapause: they do not always develop immediately upon fertilization but need a further relevant stimulus. Indeed this is true of many eggs which are "laid": that of the birds effectively has a diapause at the blastoderm stage and both this and the subsequent resumption of development appear to be active, triggered responses to temperature change; they have an all-or-none character which is not compatible with the idea that temperature change is acting in a simple direct way. The nature of the normal triggering stimulus, and its mode of action, have been discovered for the eggs of some parasites; they are found to be highly adaptive, prompt and accurate, as the precarious existence of these animals demands.

Encystment in protozoa is a suspension of animation somewhat similar to diapause, the arrest of growth again being a major feature. There is also extensive dedifferentiation, in this case. The evocation is probably not so sharply triggered or so highly coded as it is in insect diapause, the response being induced by the relevant change in external conditions itself, rather than by an anticipatory signal. Nevertheless the response is anticipatory enough: it begins before conditions are actually limiting for growth, and to this extent it is a triggered response. Encystment likewise is directly evoked by the favourable change in environmental conditions. Both responses are relatively insensitive (Hyman, 1940; Grassé, 1953) to acute experimental changes in those physical factors which non-specifically fire off the more highly evolved kind of triggered response. One possible reason is that the Protozoa have remained more primitive than the insects, in this as in many other respects, but it is also likely that the aquatic medium changes less violently than terrestrial conditions, and so permits more leisurely responses. The state of encystment itself resembles diapause in a dehydration of the body and probably in other respects. Dehydration was seen to be a feature also of ageing in metazoa and of the micro-organisms in an ageing culture (p. 114). The changes slow down metabolism in general and so avoid serious weight loss through wear and tear.

Spore formation in protozoa and bacteria differs from encystment in involving smaller bodies, usually resulting from rapid repeated fission. These bodies serve not only the function of tiding over adverse conditions but also that of aerial transport to a potentially favourable site. Possibly because of the aerial phase spore formation and germination seem to be more sharply triggered than encystment. Nevertheless there may be very protracted changes preliminary to spore formation itself; these changes occur, in lesser degree, in the growth cycle of every population of bacteria (Hinshelwood, 1957), whether or no this ends in the extremity of sporulation. The cells lose water and become less

permeable, and their enzyme activity declines markedly. If conditions permit they embark on a new cycle of growth, the length of the lag period depending considerably on the extent of the previous change towards the dormant condition.

In an old culture of microorganisms, as in many adult metazoa, the net proliferation rate falls to zero, but the gross rate is still adequate to make good losses by wear, so that the onset of a new phase of positive increase involves a mere acceleration of growth rather than a sharp change in state. There is no metastable condition and so there can be no triggered mechanism; the lag period may be relatively long partly for this reason, conditions being reversed only gradually. This may be equally true of the lag periods of metazoan cells when explanted or when provoked by carcinogens *in vivo*.

There is some knowledge of the kind of control mechanism involved in these non-triggered responses, in vertebrates. A factor, shown experimentally to depress growth, is present in the serum of birds and mammals and increases in concentration or in potency with age (Grimm, 1949); the increase proceeds in parallel with the decline in general growth rate, of which it may be the major cause. This serum factor is to be distinguished from the organ-specific inhibitors (p. 356), which are concerned rather with differential growth between organs than with absolute growth. There is some evidence (Bullough, 1962; Hemingway, 1960) that the corticosteroid hormones (p. 369) are responsible for the general inhibition of growth in adult mammals and that they may constitute the serum inhibitor in question. Hemingway suggests that growth may be resumed if there is an adequate decrease in the ratio of the amount of the corticosteroids to that of APGH (p. 365), whether due to a decrease in the one or an increase in the other. It is further suggested that a reduction in the ratio locally may release local growth, as in tumours and in regeneration blastemata. In this last instance the hypothesis is that damaged cells release oxidizing substances which destroy the steroid hormones locally and temporarily. The net result of destroying the steroid inhibitor would be much the same as that of the equally hypothetical wound hormone (Needham, 1960), which is visualized as a positive growth-promoter, however.

The theory seems less plausible as an explanation of changes in local than in general growth. A circulating hormone would seem to be in danger of general rather than local depletion by a destructive agent liberated from a wound. Although the hormones do show actions restricted very often to local target organs (p. 350), there is no good evidence that in such cases the hormone is destroyed or inactivated by the other organs, as this theory might seem to imply. Beryllium salts rapidly destroy or inactivate the wound hormone (Needham, 1941; Thornton, 1949) but at present there is no evidence that they are likely to destroy the postulated oxidizing agent.

The wound hormone is produced very transiently and therefore seems to be essentially a typical triggering agent. In consequence, perhaps, the regenerative response is much more prompt than it would be under the sole influence of a

decrease in concentration in the blood of the organ-specific factor(s) of the amputated organ(s), and its significance is not related to this more systemic type of growth-controlling agent. The triggering seems particularly clear when diapausing pupae of insects are injured (Shappirio, 1960); their response has all the features of a local breaking of diapause. After wounding in a mammal, the adrenal cortex hormones are involved in a systemic *general alarm reaction* (Selye, 1950, 1955) more than with the local processes, though these are affected (Needham, 1960). The systemic response also presumably must be regarded as triggered in this case; the vertebrate hormonal system can be switched in this way but at other times it operates more gradually. Control of growth by APGH is deployed with much the same acumen as that of the insect GDH; it was seen (p. 367) to affect cell division, the synthesis of proteins and nucleic acids, and other key components of anabolism. Steroids likewise show acuminous deployment; they control the uptake of materials by the cell and the balance between anabolism and catabolism for the main categories of biological material (p. 370). They are also effective solvents of NA (Henry and Stacey, 1946). In this connexion it may be significant that the arrest of growth in an oocyte is correlated with the disappearance of its NA-rich nucleolus (Pollister, 1954), and that steroid hormones are produced in the ovary.

It is not certain that the master control of growth in mammals depends only on the balance between these two hormones or even on the hormonal system alone. The cells themselves seem to become progressively less resistant to inhibition, so that their growth is arrested by a concentration of serum inhibitor which does not halt the growth of cells from young individuals (Medawar, 1940). Indeed, when grafted into an older individual the latter may grow more rapidly than *in situ* (Twitty, 1955), which probably indicates also a higher titre of the growth-promoting hormones in adult serum; the young cells are more sensitive to the promoter and the old cells to the inhibitor. The net result is much the same as their respective responses to their organ-specific factor (p. 356). There is a further example of double assurance (p. 343) in this increasing inhibitory power of the serum, coupled with increasing inhibitability of the cells. There may in fact be triple assurance, that is to say the cells possibly take up increasing amounts of the inhibitor and so help in their own growth control; the evidence is that extracts of old, but not of young heart tissue contain a factor which inhibits the growth of tadpoles (Kotovsky, 1931; Comfort, 1956). Simms and Stillman (1936) found that an inhibitory factor does accumulate in adult tissues but it is not known if this is derived from steroids or other circulating hormones.

Specific growth-arresting factors have been recognized in plant roots (R. Brown *et al.*, 1952) and a promoter-inhibitor pair possibly operates in duckweed (Ashby and Wangermann, 1954). In a clone of the latter, growth rate decreases progressively over the generations, as over the cell-generations in the metazoan body. In plants, however, a single hormone, for instance the auxin, indole-acetic acid, can both promote and inhibit, according to concentration. Moreover,

owing to a varying sensitivity in the different organs, a single concentration may inhibit root-growth while it is promoting that of the shoot (Thimann, 1960).

Interaction between the GDH and allatal hormones of insects (p. 381) appears to control the course and termination of growth in much the same way as the postulated hormonal mechanism in mammals. However, the mechanism is adapted to accept the triggered interruption of diapause at almost any stage of the growth programme, in different species, and some mammals have a possible counterpart to this in the phenomenon of hibernation. As in diapause, both growth and the activity of the endocrines is radically modified at this time. The general mechanism accepts other modifications also, as when the mammalian gonadal hormones induce a spurt of growth at puberty.

There are two leading questions at this point: How does the triggering of development in the egg relate to the hormonal control of later growth and how is the progressive shift in hormonal balance throughout the life programme itself controlled? The endocrine glands of the embryo are not functional until a relatively advanced stage. Moreover the embryo's growth is insensitive to APGH and other hormones until that stage, so that there can be no control by maternal hormones carried over in some way. It is possible, as already suggested, that steroids arrest growth in the mature oocyte, and that they must then be inactivated at fertilization, but there appears to be no definite evidence about this. It seems more probable that there is a sharp distinction between the initial triggered regime and that which gradually develops subsequently. This has already been considered in connexion with the organizer hierarchy (p. 390). It was pointed out (p. 16) that in addition to the final spurt of growth at puberty mammals usually have two cycles of growth (Thompson, 1942, p. 160). The first occupies only a fraction of prenatal life so that the onset of the second might well mark the transition from the initial, to the definitive mechanism based mainly on the endocrines.

The control of the steady shift in hormonal balance, subsequently, remains a major problem. It seems that a particular hormonal ratio must be closely bound to a particular body size so that growth can be halted at any stage by diapause and other incidents, and yet resume quite normally, and go on to produce an invariable final size. In some insects diapause is facultative, according to external conditions, but whether diapause occurs or not, growth follows the same programme, within its limits of error, and gives the same final size and form. In most insects the duration of diapause is variable, again depending on external conditions, but still the programme is only delayed and not otherwise disturbed. It is most probable that the shift in hormonal balance depends on a genetically determined pattern of differential growth, and so of differential productivity, of the relevant endocrine organs themselves (p. 135). This should prove to be closely related to instantaneous body size, since it is itself part of the growth programme. It is significant that gross abnormalities of growth, giving dwarfs and giants, in practice are usually due to hormonal aberrations, as experimental work has shown for insects, amphibia and mammals.

In answer to the questions raised at the beginning of the chapter, therefore, it may be said that the endocrine system acts as a master control throughout most of the life programme of the higher animals, except at the outset, when the embryo's organizer system is the integrating control. Both show a cascaded deputization of control to lower levels of size and organization. The internal master control, genetically determined, is absolutely controllable by those external agents capable of acting as triggering stimuli, switching growth on or off in an all-or-none fashion. However, after switching off for a varying period the growth programme may be switched on again, precisely as though there had been no interruption; the execution of the programme therefore depends entirely on the internal master control. External triggering agents act simply in a permissive fashion. They remain quite detached from the growth system and the latter has been selected for ability to respond to those which are useful, as detached agents. Even so the mechanism of triggering remains an important and intriguing mystery, with potential information to yield about the growth process itself. Not all growth cycles are triggered at their onset and cessation, but these may nevertheless have a triggered diapause during the cycle.

As a result of studying it under the specially favourable conditions of diapause in insects, it appears that regeneration involves a complete, supernumerary but local cycle of growth, triggered by wounding. In regeneration we are particularly reminded of the sharp distinction between growth and differentiation as components of development. In the present context this prompts the final major question: how far is the control of differentiation bound up with that of growth? This will be considered in the next chapter.

## CHAPTER 27

### *Growth and Differentiation*

It was necessary at the outset of the analysis (p. 2) to distinguish between growth and differentiation. It is therefore fitting to conclude the final synthesis by returning to the relationship between the two, particularly in the way they are controlled. For this purpose, only the general aspects of differentiation can be considered: specific differentiation processes are the province of the morphogeneticist, a field much too vast even to be mapped on a small scale here.

It was shown in the introduction that a great deal of what is popularly regarded as differentiation, in fact is differential growth, for instance a change in the proportion of the various types of cell, and that true differentiation should be defined as a purely qualitative change, the rearrangement or the change of units, so excluding any mere addition of units. It is usually manifest as a progressive divergence in character between one body region and another (Fig. 27.1), and is both spatial and temporal in nature therefore; it is sometimes purely temporal, however, as when an entire and quite homogenous population of molecules, or other units, is converted into, or replaced by, an equally uniform population of a new type, without any spatial variation. This is more usually a replacement, a change in a pathway of biosynthesis (p. 3), than a direct conversion of molecules and so is largely a growth phenomenon. This is often true even of spatial differentiation.

It is clear, therefore (p. 3), that a sharp distinction between growth and differentiation presents some logical difficulty, in addition to the practical one arising from their close integration into the process of development. The logical distinction should be most easily made at the molecular level, but at present there is little critical information here. At higher levels, using available criteria for distinguishing the two components, it has been possible to demonstrate a considerable degree of independence between them (J. Needham, 1942, p. 507 ff.) which encourages further analysis along these lines. Anidian chick blastoderms are abnormalities which show cell proliferation but never differentiate into an embryo; neoplastic and some other cells behave similarly. Differentiation without any growth has been observed in the eye of the chick, transplanted to the chorioallantoic membrane, in whole chicks exposed to high oxygen tension, and in tadpoles given tyrosine and tryptophan as their only dietary amino acids. The action of the thyroid hormone on metamorphosis in amphibia is usually regarded as mainly differentiative and the common type of human dwarf, due to deficiency of pituitary hormones (Fig. 23.1), shows full adult differentiation with virtually no growth after infancy. Some genetic

mutations affect differentiation without any evident action on growth (Villee, 1942). Mycetozoa commonly have a sharply segregated period of growth and proliferation, followed by one of pure differentiation of the fruiting body. There is considerable segregation also in regeneration (Needham, 1952, 1960a). Discrimination has been achieved experimentally also: in *Drosophila* quinones

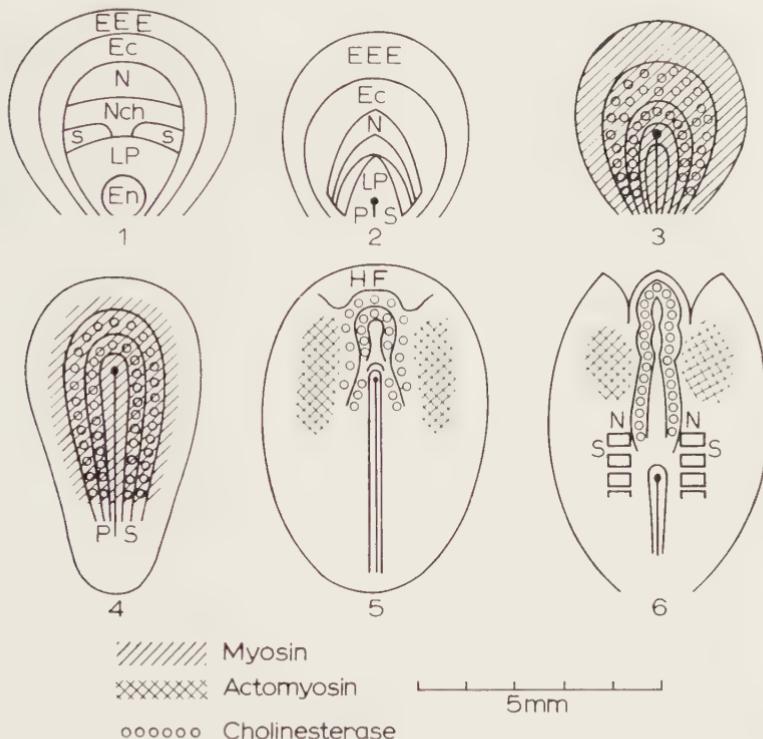


FIG. 27.1. DIAGRAMS OF THE BLASTODERM OF THE CHICK, IN SUCCESSIVE STAGES OF EMBRYOGENESIS, TO SHOW THE DISSEMINATED ORIGIN OF MYOSIN, ACTOMYOSIN, AND CHOLINESTERASE, AND THEIR PROGRESSIVE RESTRICTION TO THE HEART AND NEURAL TUBE RESPECTIVELY

*Ec*, ectoderm; *EEE*, extra-embryonic ectoderm; *En*, endoderm; *HF*, head fold; *LP*, lateral plate mesoderm; *N*, neural plate; *Nch*, notochord; *PS*, primitive streak; *S*, somites. All presumptive in the early stages.

(Based on Ebert, 1954b, and Hermann, 1959)

inhibit growth but not differentiation, while the converse is true for colchicine.

This degree of independence and dissociability has been taken to support the further view that in development there is actually an inverse or reciprocal relationship between them, if not a directly antagonistic one. There is some support for the idea of a general incompatibility at least. *In vitro* mammalian cells appear not to grow and proliferate until they have dedifferentiated to

some extent (p. 89) and this is true also of the cells of regenerating structures. As growth slows down there is increasing redifferentiation of the progeny. Cancer cells grow at a rate inversely proportional to their degree of differentiation. The skin and other tissues continue to proliferate in adult life, but from undifferentiated cells. It has therefore been suggested that differentiation in some way inhibits growth (Robertson, 1923), but there is at present no clear evidence for this, for instance that differentiation produces positive growth-inhibiting agents. Indeed extracts of nerve cells, one of the most highly differentiated types of cell, are in fact among the most powerful in growth promotion (p. 91). If growth were to resume spontaneously in a differentiated tissue, its differentiation should be automatically diluted down, and this has been suggested in explanation of its apparent reversal in certain instances. On the other hand there is no doubt that true differentiation must soon be halted, for lack of material, once growth has ceased, and it may be more true that growth controls differentiation than *vice versa*. This control is not antagonistic.

The two are not so sharply segregated in time as is sometimes supposed. Many cells grow and proliferate during at least the intermediate stages of differentiation (Huxley, 1932) and cell division does not cease abruptly before differentiation begins, as sometimes inferred (Løvtrup, 1959, p. 110). Indeed neurons *in vitro* are said to be able to divide even as fully differentiated cells (Fischer, 1946). Functional hypertrophy (p. 4) is a growth of fully differentiated tissues, and in short the two processes overlap considerably in ontogenesis. The fact that differentiation is maximal at the end does not necessarily mean even that its rate is maximal when growth has ceased. Visible differentiation is the integral of the actual process, just as size is the integral of growth: the actual rate of differentiation may be maximal much earlier. Confusion arises from the fact that the same term covers both differentiation and the integral of the process. Possibly the term "differescence" might be suitable for the former. The maximal rate of differescence certainly seems to be later than that of growth but this may be largely because its *relative* rate is enhanced as growth slows down. If differescence is a change in material already synthesized then it will be expected to post-date growth much more sharply than if it is a change in the actual pathways of synthesis (p. 3).

Schmalhausen (1931) held the view that there is a direct and not an inverse correlation between the two, and gave the relationship a quantitative formulation. His main assumption was that the differescence rate at any stage is proportional to the growth rate of the material remaining undifferentiated, implying a close parallel between the courses of the two. Others believe that the rate of differescence is constant throughout and that, as already suggested, it merely appears to increase as growth slows down, and to reverse when growth resumes. For instance, the amount of pigment per cell, as an index of differentiation in the iris, decreases when iris cells are first explanted, and resume their proliferation; later, as growth slows down, the concentration of pigment increases again (Fischer, 1946). Of course these same effects would be obtained

even if the differescence rate varied somewhat, and there is no *a priori* reason to believe that it is any more constant than growth rate. Most probably both proceed in cycles, a cycle of growth immediately preceding one of differescence. Thus in the development of the individual organs, which offers a simpler system than the whole body, a bout of cell proliferation precedes differescence (Robertson, 1923) and this is well illustrated in the optical vesicle of vertebrates (Bonner, 1952, p. 131). Again, there is a great increase in mitotic index in grafted embryonic tissue just before it is induced by the host to differentiate into a secondary neural tube (J. Needham, 1942, p. 177).

Growth and differescence therefore are asynchronous, but they are directly rather than inversely related so that when the lower Metazoa degrow they also dedifferentiate (p. 29). In encystment, again, dedifferentiation is associated with the arrest of growth. Again, the interstitial cells of coelenterates and other animals remain dormant, but also undifferentiated (von Bertalanffy, 1960); when they do proliferate differentiation soon follows, in the progeny. If the two were really inversely related there should be occasions when differentiation promotes degrowth: in fact, however, there are examples of tissue interactions in which differentiation prevents resorption (p. 437).

The phylogensis of the two components should help to clarify the relationship between them and this can be studied to some extent in primitive extant organisms. Even viruses (p. 153) have a differentiated structure; in the bacteriophage type this is largely broken down during the phase of growth and multiplication to be restored only in the mature transmissive bodies. Before this time the material may increase a hundredfold or more; here the two components are sharply segregated, therefore. In some other viruses, however, the segregation is less complete; the initial dedifferentiation is less pronounced and redifferentiation follows closely on growth at each stage. Bacteria (p. 111) might be said to show a sharp segregation, to dedifferentiate at the onset of a cycle of population growth and to redifferentiate towards the end. Certainly at the beginning there is increased hydration and other changes characteristic of actively growing cells, while luminous bacteria provide evidence about redifferentiation; they complete 30 to 40 per cent of their proliferation as a population before differentiated luminous material is detectable (Harvey, 1952, p. 25). The amount of this is maximal at maximal population size, when growth rate has fallen to the maintenance level; presumably the two are now precisely in step, differentiation keeping pace with new production. These features once more emphasize the parallel between a population of micro-organisms and a metazoan body; the changes apply to the population as a whole and not to each cell cycle. They differ from those in a developing metazoon in being stage-specific only: there is no spatial differentiation except in certain special cases (p. 114).

There are in fact certain qualitative changes even during the individual cell cycles of a bacterium, but apart from those concerned with cell division they may be relatively unimportant. However, many of the larger and more

complex of the Protozoa, the Ciliata in particular, have very definite cycles of de- and redifferentiation in each cell cycle (Calkins and Summers, 1941; Hall, 1953). Differentiation of the organelles for the two daughter individuals may precede both the dedifferentiation of the parental organelles and fission (neotomy or paratomy) or it may follow both (architomy). In either case growth of the new daughter organelles occurs mainly after their differentiation. In some ciliates the daughters share and retain parental organs and regenerate the remainder after fission; once more the new daughter organelles are largely differentiated before they grow. This is possibly a reversal of the phylogenetic order since differentiation arose mainly as a division of labour between parts, and at each stage this would presumably be possible only after a certain minimal increase in size (Huxley, 1912) to provide adequate material. However, no doubt one of the advantages conferred by a useful division of labour would be an increase in general efficiency, permitting further size increase; this is probably reflected ontogenetically in the post-differentiation growth of the Protozoa and in the auxanodifferentiation (Huxley, 1932) of metazoa. In any event, changes in the phylogenetic order are known to occur as ontogenetic adaptations. Probably because of the high degree of differentiation in the ciliate Protozoa, entirely within a single cell, it is necessary to segregate rather sharply the events of differentiation, dedifferentiation, growth and cell division; segregation *eo ipse* seems more important than any particular order. Some degree of independence between their respective control mechanisms is to be expected, therefore.

Turning to this aspect, the control of differentiation, it seems that if regeneration may be regarded as typical of morphogenesis in general then the nervous system is not necessary for its control (Singer, 1960; Needham, 1960a), as it is for growth. This is somewhat surprising in view of the facts that nervous controls are essentially local, and that differentiation is so essentially spatial, but it will be recalled (p. 122) that end organs specify their nerves rather than *vice versa*. By contrast hormones, although systemically distributed, do control local differentiations. Those of the thyroid and the insect prothoracic gland are perhaps the most important of known differentiation hormones. The gonadal hormones control sex-specific differentiation, though here, and no doubt in most cases, it is difficult to decide how much of the effect is pure differentiation: the hormones also control local growth (Chapter 23).

Early differentiation, like early growth (p. 395), is controlled by the quite independent mechanism of the embryo's organizer system which is responsible for topological changes and other very dramatic components of differentiation which Dalcq (1960) calls morphochoresis. In addition, there appears to be in mammals an overriding master control of maternal origin, since *in vitro* embryos grow but do not differentiate (Schechtman, 1955). This is not true for bird embryos and may be peculiar to viviparity. Organizer control is somewhat unilateral in action though perhaps not to the extent that early work seemed to indicate. The unilateral property depends on quantitative differences in

activity, including growth, the more active region having more influence on the differentiation of the less active than *vice versa*, and being known as the organizer in consequence. This quantitative basis for organizer action is particularly evident in the regeneration of the lower Metazoa (Child, 1941; Bröndsted, 1955) but is well recognized in embryogenesis also (Brachet, 1950). It constitutes further support for the view (p. 434) that growth phenomena may control differentiation.

Control of differentiation by the main organizer is described in the classical works on experimental embryology. Studies on the lower-grade organizers, in general are more recent, the work of Toivenen (1950) being a landmark. Grobstein (1954) and others have studied the activity of lower-grade organizers *in vitro*, and there has been considerable work on simpler associations of relatively pure tissues, *in vitro* (Fischer, 1946). At this level it is possible to gain some insight into the way differentiation is controlled. Pure cultures of one cell type will grow and proliferate under conditions which cause an association of two or more types to differentiate. Here, therefore, there is some positive indication that differentiation itself may inhibit growth, but this is by no means certain: the growth inhibition may come first. As already noted (p. 435) differentiation certainly does not promote degrowth, but rather prevents it: when intestinal epithelium and connective tissue are grafted singly into the anterior chamber of the eye, serving as a culture medium, they are resorbed, but if grafted together, they persist and differentiate. Contact between cells of different type therefore promotes differentiation rather than growth, and the nervous tissue would seem to be exceptional (p. 353) in this respect. There is a biological rationale for this kind of control of differentiation, by local contacts, based on the consideration above (p. 436) that effective division of labour demands adequate cellular material among which to divide the labour. One part of an embryo or of an organ, therefore, does not differentiate except in the presence of adequate material undergoing the complementary differentiation. Within limits cell associations are capable of regulation, that is of readjusting the fate of individual cells so as to give a reasonable balance between the numbers differentiating in each direction (Hadorn, 1961).

Little is yet known of the chemical processes involved in the interaction between tissues, but one very instructive example concerns the cornea. Here collagen formation in the dermal stroma appears to depend on the ability of the epidermal epithelium of the cornea to oxidize the lactic acid produced by glycolysis in the stroma (Hermann, 1959). This level would seem to be the most promising for further research.

For differentiation proper, cell division itself is relatively unimportant, though the chromatin diminution and chromosome elimination of some embryos (p. 391) is incidentally related to nuclear division. Cell division is essential also for completing the cytoplasmic segregations which play an important part in differentiation (p. 392). Differential growth depends very often on the orientation of division spindles (p. 85).

At the intracellular level one of the outstanding features is that nucleic acids are much less important for differentiation than for growth (p. 323). Also in sharp contrast to growth, differentiation may require little new material from outside, in keeping with the idea that true differentiation is purely a rearrangement of existing material. If the gonad of a young animal grown *in vitro* is starved of amino acids it continues to differentiate but does not grow (Wolff, 1957). This is true also of simpler tissue preparations (Lovtrup, 1959). A similar response is often evident following food deprivation to the intact growing animal or plant; although stunted it may mature and produce young. Not infrequently the productive activity, for instance flowering in plants, is greater than when liberally fed (p. 281).

The extent to which differentiation is simply a rearrangement of existing material might also explain why some workers have found no change in amino acid composition or a.a. requirement at the onset of differentiation (Nickerson, 1959). Others, however, have detected such changes (Gustafson and Hjelte, 1951), and this is in keeping with Gudernatsch's classical demonstration (p. 288) that some amino acids, tyrosine, phenylalanine and tryptophan in particular, are very specific to differentiation, and others to growth. Further support for his results has been provided by the discovery of Wilde (*see* Lovtrup, 1959) that the addition of phenylalanine to a culture of embryonic ectoderm cells induced them to differentiate into pigment cells; otherwise they developed as ordinary epidermal cells. Again, in the growth cycle of bacteriophages there is a tryptophan-mediated process occurring at twelve minutes from the onset, and this is during the differentiation phase (Cohen, 1949).

Differences between the controls of growth and differentiation extend to the action of inorganic substances. In marine bioluminescent bacteria 1·5 to 2·0 per cent of NaCl is optimal for growth but 2·7 to 3·5 per cent for the synthesis of the luminescent material (Harvey, 1952). Again, however, it is not certain that the distinction is between pure growth and pure differentiation, and this problem is as critical as it should prove fruitful for further research.

In general embryologists probably regard development as mainly a process of differentiation, while the auxologist may incline to the reciprocal over-emphasis. Much benefit could result from a more critical delineation between the two components. In those cases where a clear distinction has been made between them, significant differences in properties and in control mechanisms also have been found, and we may look forward to further extensions of this analysis.

It seems a reasonable generalization that the processes of growth and differentiation are directly rather than inversely correlated, complementary rather than reciprocal or even antagonistic. This is also the phylogenetic relationship. They are asynchronous, however, to a degree which may depend on the precise character of differentiation, as well as on the particular biological requirements of each case. In some instances there is virtually no overlap in time. Resumption of growth automatically reverses differentiation by a

dilution effect, and it is not certain that dedifferentiation must absolutely precede regrowth. Growth can occur even in highly differentiated cells and positive antagonism between the state of differentiation and the process of growth has not been proved. There is more evidence that growth controls differentiation than vice versa. The mechanisms controlling growth and true differentiation have considerable independence: this would be expected on the general principle of multiple assurance (p. 343), as well as on phylogenetic grounds.

## CHAPTER 28

### *Conclusion*

In his great work, *On Growth and Form*, Sir D'Arcy Thompson (1942) wrote "This book of mine has little need of a preface for indeed it is all preface . . ." and for the same reason he was reluctant to attempt a formal concluding chapter. His modest apology can scarcely hide the fact that the breadth and scope of his subject matter would have made an integrating conclusion very difficult. The present study is restricted to the growth aspect, and it has had the advantage of further years of fertile research, so that there is less justification for not attempting a concluding chapter. Also there is the challenge of another author in the field, that books are abandoned rather than finished. Certainly the task is not easy, and inevitably there is the conviction that while the facts already presented may weather the years, present attempts to roof them with generalizations may not. These are necessarily coloured by personal interpretations and by contemporary fashion, as well as being based on evidence which is still fragmentary.

A concluding chapter should show not only what is now established but also what is still unknown, or more specifically, should indicate the most urgent and the most promising lines for immediate research. This is perhaps not very difficult in general terms but the tactics, that is asking the right questions in the best order, present more difficulty. Here the corrective and directive effect of research progress itself is indispensable and it is not an enterprise for detailed pursuit here.

It may be taken as established that growth is fundamentally similar in all organisms. Certainly there are some features peculiar to the growth of particular groups of animal but it has been possible to study most aspects as a common property, at least of all heterotrophes. They all show the same kind of relationships between growth and differentiation. All show similar, if also rather non-specific, sigmoid curves of size against time, with a phase of exponential increase, followed by one of exponential decrease in gross growth rate, that is to say a common mode of progress through the cycle of growth from one steady state period to another. Organs, populations of cells, populations of microorganisms and individual cells also have this same type of growth cycle. The various spatial and spatiotemporal patterns of growth occur widely in many different groups of animal. Cell growth and cell division are so similar in all that this similarity is taken for granted, and this is equally true of the nature and behaviour of the cell organelles. Possibly these similarities

are taken too much for granted. At the macromolecular level there are great similarities in the methods of synthesis of many different protein materials. At progressively lower levels, in the synthesis of polypeptides, nucleic acids and smaller molecules the uniformity is even greater.

All organisms have exploited the same few monosaccharides and their derivatives, the same few glycerides, phosphatides, steroids and isoprene polymers, and the same amino acids, nucleotides and B-vitamins. Occasionally, especially among microorganisms, certain more unusual constituents are common, but this seems to imply simply that the higher organisms have narrowed the common field of exploitation. All have mainly D-sugars and L-amino acids.

In one sense, therefore, biosynthesis is very non-specific, but in the sense that only a few of the many possible organic compounds are exploited by living organisms it is highly specific. It is highly specific to life but very non-specific among living organisms. Taxonomic specificity therefore seems to rest entirely on the way these common basic units, particularly among the a.a.s and nucleotides, are put together to make fabric.

There is also essential similarity between all heterotrophes in the mode of control of biosynthesis by raw materials, by transerase enzymes and by nucleic acids, and in the interrelationship between cell growth and cell division as a factor in the control of growth. Above this level of control, however, there is more significant variety. In microorganisms external factors impinge on the growth mechanism at the cell level but in metazoa there is above this an intermediary hierarchy of controls. Local interactions between tissues, by diffusing chemicals, may have evolved first, then control by a nervous system and finally, when a circulatory system had appeared, control by hormones and other systemic agents. The various phyla of the Metazoa have reached different levels in this evolution; just as in their general physiology so in their growth control mechanism, the higher metazoa have attained a degree of integration at least as efficient as that of the most highly evolved of protozoa, the Ciliata. They have also come to respond at least as promptly and efficiently to external agents. There is a degree of convergence in the independently evolved systems of the different groups of metazoa, for instance the hormonal systems of mammals, insects and crustaceans. There may be convergence at lower levels, also, but this is less easily demonstrable at present.

Metazoan integration has been achieved largely because individual organs and parts of the body have sacrificed much of their autonomy in the common interest. The subordination breaks down in such instances as neoplastic growth. In consequence of the subordination, the growth requirements of the individual organs tend to be greater than those of the body as a whole, because they have delegated some of their synthetic powers to central factories such as the liver. In some cases, however, their demands are less because of the restricted functions of the cell, and in general they are very variable. *In vitro*, therefore, the growth properties of a tissue may appear very anomalous. Some of these are not easily explained, for instance (p. 90) insensitivity to hormones which

control the same tissue *in vivo*: possibly the correct balance of all hormones is required for an effective response.

A similar subordination of components to the whole is seen within the individual cell, of both microorganisms and metazoa. The whole cell is essential for sustained growth (p. 336), and synthesis by isolated organelles soon ceases. The cell is the smallest unit which will grow autonomously, since viruses are not known to grow independently of a living host cell. The ability of viruses to tap the host cell's machinery for synthesis in this intimate way is one of the most powerful pieces of evidence for a close kinship between the growth mechanisms of all organisms.

The second main conclusion is that growth, in the usual sense of a positive size increase, is only a special case of a general phenomenon which enables living organisms to balance wear by repair, at all levels and all stages of the life cycle, and to maintain themselves as open systems in a permanent steady state. To quote Claude Bernard (von Bertalanffy, 1957, p. 7), "Synthetic activity for maintenance . . . is . . . basically the same as that by which an animal heals wounds or grows or reproduces. Organic synthesis, reproduction, regeneration, wound healing and integration are only various aspects of a single phenomenon." It is tempting to add that this single phenomenon effectively is Life. All physiological work must be balanced by rest and recovery, the latter being slightly differential to permit functional hypertrophy and disuse atrophy (p. 4). Orthodox growth, a rapid increase in size of the bodies formed by reproduction, is necessary to balance the loss of whole individuals by the kind of wear which occurs at this level; the egg grows in a controlled manner and re-establishes a steady state at the normal definitive size of the new individual.

Growth *sensu strictu* is only the more kinematic phase of the unitary life programme: the later phase, of maintenance, is possibly even more remarkable than the growth phase, since it would seem to be rather easier to maintain a positive balance in favour of anabolism than a precise steady state. This demands a complex system of negative feedback devices and is the crowning achievement of living organisms; they are the outcome of natural selection for just such self-perpetuating steady state systems. The view that growth is the essence of life is unquestionably correct in the sense that anabolism continues at all times, in all active organisms, but it might be considered incorrect in the sense that anabolism is only the one half of the balance. The usual view is that, since catabolic disintegration represents the major natural obstacle which life has to overcome, therefore life itself should be equated with anabolism alone. For individual species this might be satisfactory but in a wider context it is an incomplete view (Needham, 1959); anabolism alone would be deleterious, and even as disastrous, biologically, as unchecked catabolism. Life is a strict dynamic balance between the two. In spores and other dormant bodies the balance is, of course, negative, but only very slightly, because catabolism is minimized so efficiently (Keilin, 1953, 1959).

It should be admitted that there probably are some significant differences

between the phases of positive growth and of maintenance, for instance in respiratory metabolism (Chapter 16). However, these may depend on quantitative rather than strictly qualitative differences and this is equally true of the difference in metabolic turnover (p. 191). It is clear from the study of mammalian hormones, in particular, that there is a common control of growth and maintenance, and that this acts on both anabolism and catabolism, shifting the balance between the two. The pituitary growth hormone, APGH, for instance, seems to act mainly by reducing protein catabolism (p. 189). The growth programme passes smoothly from the one phase to the other and in some cases the work cycle of an organ is effectively a reiteration of the final stages of synthesis of the material of its cells (p. 5).

It follows from these considerations that as classical theory has usually maintained, growth is essentially a pristine property; equally, however, it is a biological adaptation if, as we must suppose, natural selection has operated since the very origination of life. Throughout the book there has been abundant evidence that growth and its controls are eminently adapted phenomena. The wisdom of food selection (p. 282) is one instance. The rate of growth is another: it varies enormously among animals. Few grow at the maximal potential rate, but all at a rate which is adapted to the particular requirements of the species. It may therefore be a mistake to try and speed natural growth (p. 279). The repression of growth is usually just as much a positive adaptation as its promotion: certainly arrest is not due simply to the fortuitous absence of promoting conditions (Chapter 26). Consequently non-growing eggs and individuals are often in a metastable condition from which a light triggering stimulus can displace them into a new cycle of growth. Such on and off switches play an important part in growth control.

Among the many other impressive instances of adaptation is the more economical use of food when intake is restricted. There is less growth retardation during the period of restriction than would be expected, and on refeeding at the original level, 73 per cent of the food may be used for weight increase compared with 26 per cent before the restriction. In rabbits an intake previously inadequate even for maintenance may cause a weight gain of 56 per cent after a 17-day fast (Robertson, 1923, p. 240). These adjustments probably operate mainly through the hormonal system. Another very interesting adaptation is illustrated by the mammalian skin: restriction of food leads to a slower proliferation of epidermal cells but there is also a compensatory reduction in the rate of scarification, and so the epidermis maintains its normal thickness (Bullough, 1952).

Another instance is the rebound acceleration of growth after a period of enforced retardation. Children show this (Fitt, 1941), so that the paediatrician need not worry unduly about minor setbacks. Compensatory rebound is a familiar phenomenon (Comfort, 1956, p. 155; Wilson and Osbourn, 1960), even *in vitro* (Medawar, 1942). Buchanan (1938) recorded a rebound acceleration of growth after cooling, and after KCN inhibition, and Wilson and Osbourn

(1960) cite many examples after food restriction. The rebound may begin while the causal agent is still operating, as in the responses of growth in fish, both to a deficient and to an excessive diet (M. E. Brown, 1957). Barnacles of a species of *Chthamalus*, which is normally intertidal, grow more rapidly than is normal, when first immersed continuously, but later the rate becomes subnormal, and the normal adult size is reached in the normal time (Barnes, 1956). These cases show an interesting analogy to the escape and accommodation phenomena in neurological responses.

Perhaps the most outstanding example of adaptive plasticity is the insurance of a constant species-specific adult size. The biological need for this is illustrated by the fact that although some animals, and a larger number of plants, from time to time produce polyploids, which are correspondingly larger than normal and survive because of other selective advantages, they do not always maintain the giant size; selection eventually secures a polyploid strain with the original body size. Within individual ontogenies final size has a degree of independence of factors which affect the actual course of growth, its rate in particular. The most acceptable of the mathematical definitions of growth (p. 14) have recognized this and show final size independent of all other variables and all but one of the parameters which enter the definition. Thus the logistic relation gives a gross growth rate  $dx/dt = (k/a) \cdot x \cdot (a-x)$ , where size,  $x$ , and time,  $t$ , are variables and  $a$  and  $k$  are parameters. This integrates to give a limiting size,  $a$ , independent of  $t$  and  $k$ . The latter is the growth characteristic of the particular case, the "velocity constant" of Robertson (1923, p. 39), and may vary between individuals of different genetic constitution, and under different conditions; the relation recognizes that these need not affect final size. The experiments of McCay (1952) were among the most dramatic in this connexion; with restricted feeding, rats which normally take about 300 days to reach maturity grew slowly for 900 days to attain very nearly the same adult size. The initial size of the egg of an oviparous animal is another factor which has relatively little effect on adult size though it affects hatching size (p. 398). Insects with facultative diapause (p. 382) have the same imaginal size whether diapause is long or short.

The regulation is not absolutely infallible. Grossly underfed rats eventually lose the power to complete growth when refed, and grossly inhibited regenerates never attain normal size (Needham, 1941). This is true also of population size in a culture of microorganisms (Hinshelwood, 1944). Precisely because biological responses are flexible they cannot be perfect (p. 343). Sometimes a higher growth rate than normal leads to a subnormal final size (von Bertalanffy, 1957), for instance as a result of high temperature or of an excess of one of the hormones. Even man is said to develop more rapidly and reach a smaller final size at high environmental temperatures (Beeche and Picado, 1940). In other instances (Thompson, 1942, p. 268) acceleration results in a supernormal final size (p. 280). In these cases possibly  $k$  is caused to change during the actual course of the growth cycle. Hormone imbalance can cause a very considerable

aberration in final size (pp. 365, 385) and this only serves to emphasize the precision with which hormones normally control growth and size.

Among the many other manifestations of adaptation is that of multiple assurance (p. 343), which prevents any one aberration of normal conditions from completely disorganizing growth. It demands flexible connexions between components of the process, and a degree of independence between them. In some way this flexibility co-exists with the high degree of integration which guarantees the constant final size, and its subsequent maintenance. Flexibility, possibly of the same general type, is shown also (p. 364) by the variable action of hormones, according to circumstances. In the mammals, as already emphasized, the complete anabolic-catabolic balance is controlled at the systemic level by hormones, and each has a very comprehensive set of actions. It is mainly because of the multilateral and manifold interactions between these that individual hormones can change their effective action under the different circumstances, even to the extent of complete reversal.

This is one of a number of kinds of paradox seen in growth responses. Paradox is necessarily one of the outstanding characteristics of adaptive mechanisms because life itself is an entropic paradox (p. 6). Three other types of paradox were considered in Chapter 25. Perhaps the most striking example of the most enigmatic type, the counteracting response, is the actual retardation of growth by a high level of nutrition (Simmonds, 1948), or perhaps more correctly an acceleration by underfeeding; the presumed biological reason is that food shortage may presage famine and it is necessary to produce eggs to tide over such a period. Biological systems evidently are much more complex than inanimate ones, and a quotation from Davenport (1899, vol. II, p. 331) is rather appropriate here: "Hitherto we have regarded the process of growth in too mechanical a way, as though certain nutritive compounds passing into a chemical mill were inevitably transformed at a certain rate into protoplasm. . . . Growth processes are essentially vital processes . . . characterized by the same complexity . . . which we find in such a vital process as the response to stimuli."

The mistake is encouraged because adaptive mechanisms often come to have an apparent but spurious simplicity, due to natural selection operating for relatively simple ends (Needham, 1937); this is usually the resultant response appropriate to that particular external factor which is most crucial. The means to the end may be much more complex, however, and this is probably true of many growth mechanisms. The apparent simplicity might be conducive to complacency in research, and it is important to strike a balance between this outlook and one of pessimism concerning the complexity. For the most part the actual degree of complexity of the growth responses is still a matter for surmise. The growth of the wing of *Drosophila* (p. 85), of the pelage of rodents (p. 63), and other structures seems more complex than necessary, but this is probably because we underestimate the necessary complexity.

To begin the analysis the lowest grades of complication should be the most

instructive and a possible example is the use, in skeleton formation, of a calcium phosphate compound as precursor of the carbonate, instead of depositing the latter directly (p. 178). Something is already known about the mode of operation of this particular circumvention of the obstacle to a more direct mechanism. A possible second example is that of induced enzyme synthesis (p. 208). That the substrate itself should be the best inducer is the ideal biological adaptation, and there may be no more direct significance than this in the fact that it *is* the best inducer. In some cases substances quite unrelated to the substrate can induce, and induction by the substrate itself has little in common with the subsequent reaction catalysed by the enzyme. It is an acquired biological adaptation, possibly of considerable complexity, as shown by the Pollack effect and other obscure properties, and yet of disarming superficial simplicity. The synthesis of a new enzyme requires a new RNA template (p. 209), a whole new pathway of biosynthesis being put in motion, or more correctly released from inhibition (p. 211).

The next general property of growth is its necessary autonomy, in its normal context. This must be a responsible autonomy and is the basis for the observation (Hinshelwood, 1956) that individual constituents, such as proteins and nucleic acids, are not autocatalytic but that collectively they form a system which is.

“Each natural agent works but to this end—  
To render that it works on like itself.”

GEORGE CHAPMAN: *Bussy D'Ambois*

In fact certain particular syntheses do appear to have an autocatalytic property, that is to say priming or starter quantities of the product are necessary to catalyse the reaction. The best known examples are the syntheses of DNA (p. 227), glutamine (p. 293), and polysaccharides. It may be fortuitous that these are steps in the three main pathways of biosynthesis, but on the other hand there may be some positive significance in their demand for a primer. It is not clear what this might be: in principle the mechanism is unsound since either there is no reaction at all or it tends to accelerate indefinitely. Of course primer amounts of DNA are always present in the nucleus, and for the others there may in practice be a device for circumventing the requirement. For DNA the second danger, that of unlimited synthesis, is probably avoided by control at a higher level, since there is a brisk turnover of DNA in proliferating cells but none in a tissue which is not growing, however active it may be in other respects. Control of DNA synthesis is probably the key to the control of all other significant syntheses.

There is the same danger of uncontrolled excess in any spontaneous step, whether autocatalytic or not. It has been seen that protein unites spontaneously with nucleic acid (p. 232), retinene (p. 233), and other conjugants. The formation of coacervates (p. 148) and protomorphs shows that the spontaneity tends to

increase with the size of the aggregate, and could probably continue indefinitely in skeletal materials (Chapter 13). There may be considerable spontaneity even at the lowest levels of synthesis in heterotrophs, since it seems probable that amino acids are spontaneously incorporated into peptides of more than a critical size (p. 194). All these spontaneous processes demand some restrictive control. The antithesis of Hinshelwood's rule might therefore seem to be equally true, namely that many reactions are individually spontaneous but that collectively they are responsibly controlled. The ways in which pathways of biosynthesis are automatically controlled were considered at the end of Chapter 18. Controlled spontaneity must be the property of every step in autonomous systems, in their normal context, and it might be said that the essential object of biological research is precisely to show under what conditions each is spontaneous and how it is normally controlled. Those with positive spontaneity may be coupled with others having "negative spontaneity." The breakdown of ATP might be regarded as a positively spontaneous reaction, available for coupling with virtually any negative member.

At the higher levels, in metazoa, repressive control seems to be the dominant factor, and its importance is emphasized in the occasional escape, by neoplastic cells. Occasional normal dispensations from inhibitory control are shown by the growth of the bird's oocyte (p. 122) and the stag's antlers (p. 18). In metazoa the higher levels of molecular synthesis occur both in the cells and in intercellular matrices, so that the lowest level at which syntheses could be collectively controlled might be the tissue level. It may be significant, therefore, that quite small pieces of tissue isolated *in vitro*, seem to have a high degree of self-control, running through a regular cycle of growth and orderly differentiation to reach a new stationary stage. It is at this level, the tissue, that further attempts to analyse growth control could usefully begin. It has been suggested (p. 361) that systemic controls, such as hormones, act on the individual cell, because there are no control centres in organs or in tissues, but it may be merely that the methods of revealing these are at present lacking.

Although many components in the control of growth have been considered, at the various levels, much remains to be clarified in the ways they are integrated. The intuitive presumption is that an autonomous system must be essentially democratic, with the responsibilities widely, if not equally, shared at each level. There is some evidence of this in the multilateral interactions between the hormones (Chapter 23), at the systemic level, and in the manifold antagonistic and synergistic interactions between organs and tissues (Chapters 7 and 22) at the next level. Within the cell there is the mutually interdependent synergism between the various organelles (Chapter 20), and at the macromolecular level the interdependence between proteins, nucleic acids, lipids, polysaccharides such as chitin, and prosthetic groups, in various steps of synthesis. At the lowest levels of synthesis, the complex synergisms between the various transferases (Chapter 18), and the varied interactions between the amino acids (pp. 184, 289) appear to be particularly multilateral and democratic:

for normal synthesis all the relevant amino acids must be presented simultaneously (pp. 184, 189). Nevertheless, this type of control appears to be combined with one of deployed monarchial control (Chapter 26).

Considerable complexity of control is inevitable in autonomous systems of this kind, but there is, in consequence, selective value in any device which leads to simplification. One such device would seem to be the smooth continuity of particular stretches of the pathways of synthesis, and the absence of such intermediaries as oligopeptides (p. 203) and nucleotides. The synthesis of purines, pyrimidines (p. 222) and porphyrins (p. 243) proceeds in one piece and this is probably true for other pathways. Biosynthesis proceeds swiftly from one safe, stable stage to another, like soldiers using available cover to cross unsafe, open ground. The method might be called punctuated synthesis, and there is evidence for it in an aspect already mentioned, that of the control of the pathways (Krebs, 1959). Control is imposed only at nodal points of the pathways, at their ends or at branching points. Whatever is the complete explanation of this, it is clearly an economical and relatively simple device.

At a higher level, cell division provides a potential means of punctuation, though this is not necessarily always utilized (Chapter 21). Some of the oscillatory manifestations of growth are probably due to punctuation at this level. In some instances a series of pulses of growth have been traced to a succession of cell cycles, but in other cases the punctuation may be at a higher level, for instance in the periodic output of a particular hormone. Some target organs in fact seem to demand cyclic fluctuations in the output of the relevant hormone (Biggers *et al.*, 1957). It is of course necessary to distinguish growth rhythms due to this cause from those due to a rhythmically fluctuating external factor; as already indicated (p. 418), some growth systems have become so habituated to an oscillating external factor that they demand the oscillation. Any resulting oscillation in the growth itself may be incidental and not due to punctuation by internal controls. Punctuation might be expected to produce sharp discontinuities in growth rather than the familiar series of smooth sigmoid curves, but some smoothing probably results from the long pathway of intermediation between stimulus and response, in the body. This long pathway is indicated by the time-lag of 5 to 10 days before cells *in vivo* respond to a change in nutritive level (p. 93), and probably by other lag periods.

Another device which is not merely useful but virtually indispensable in an autonomous system, is the avoidance of directly reversible pathways (Krebs, 1962), at least in any one place at any one time: one-way traffic is the more easily organized, while reversible processes could lead to waste of energy and to stasis. Fortunately the necessary conditions for the two directions of the pathway, or at least of key reactions of the pathway, are so different in most cases that their segregation must have been a readily spontaneous piece of evolution. It is in protein synthesis that the segregation is most vitally necessary, and it is here that it seems most inevitable (pp. 194, 204). In the glycolytic sequence, and in the Krebs cycle, only particular steps are irreversible, a further

manifestation of punctuation. Irreversibility is probably essential also to maintain molecules as pure optical isomers (Kuhn, 1958).

While the direct reversal of pathways must be avoided, effective reversal is often necessary, for instance during starvation and locally as the prelude to regeneration (Needham, 1960a). At the molecular level reversibility may be achieved by circumventing the particular irreversible steps via alternative pathways (Baldwin, 1953). The complete pathway from a substance *A* to *B*, and back to *A* by another route, then constitutes a cycle, and this combines the virtues of one-way traffic with potentially indefinite continuance. The cycle may intermesh with others to form complex systems, the whole of which can be controlled at a single strategic point. Cyclic systems of this kind seem to be the essence of life (Needham, 1959): in them lies the dynamic permanence which alone can be equated with Life. A dehydrogenase molecule can continue to function only provided that it is reoxidized as often as it is reduced in oxidizing its substrate. An organ can only continue to function if everything expended during work is made good in the recovery phase. The individual molecules and parts change: what persists is the population of molecules and its cycle of activity. In ascending the scale of magnitude, from the basic metabolic reactions, the cycles become more complex and larger in scope. Losses of individuals are restored by the growth of new individuals; these are fed on materials from other organisms, so that this cycle may be completed very indirectly, and also much more slowly than those at lower levels. Ultimately, at the highest level, there is a single cyclically operating system, constituting the whole of the biomass; this has a period as long as a few thousand years (Needham, 1959).

If the motive power, that is to say direct or indirect solar energy, is available then biological cycles proceed spontaneously in the direction which is thermodynamically possible. There is reason to think that these cycles are not merely spontaneous but even *most probable* pathways (Needham, 1959), that is to say they represent those particular cyclic sequences which proceed most rapidly and unerringly under the prevailing conditions on earth; in consequence they are the most "viable" and so have been "naturally selected." They are the descendants of a long line of most probable systems and during the course of evolution must have added a high degree of causal integration to their basic property of spontaneity.

This does not mean that the problem of the growth cycle of an individual animal, or of any other cycle is either solved or explained away. The way in which their causal sequences work is the same fascinating problem that it has always appeared to be, in all philosophical views, but it is encouraging to believe that each system is not only fully and causally integrated but also maximally spontaneous. Such sophisticated devices as the control of the first step of a biosynthetic pathway by the negative feedback of the product of the last reaction are adequate warnings that no simple idea about evolution will alone solve the whole problem. What is required is still more factual material

and further systematic investigation of each particular aspect of the whole mechanism of growth.

### 28.1. Prospect

It is clear that there are many attractive problems for further investigation at all levels of the growth process. The main one at the organismal level is to show how the coded information of the genome is translated into the clear of development, against an environmental background which can vary considerably. At the systemic level the way in which the hormones exert their effects on the organ, or on the cell, remains very uncertain. The interactions between the hormones themselves are complex and not yet completely clear; the recent work on the salivary glands implies that there may be still unrecognized components of the endocrine system. The interactions between organs by such systemic agents as their auto-inhibitors (p. 356), and between organs and tissues by direct contacts, offer a particularly fascinating field for research. The relationship between cell growth and cell division is still a major problem. Within the cell the main problems are spatial, or morphological, as biochemists are the first to emphasize (J. Needham, 1936; Peters, 1937, 1949); these problems have come much nearer to solution since the advent of the electron microscope. In view of the close synergism between the B-vitamins, and of their co-existence in all typical cells, it would be reasonable to look for a correspondingly compact morphological organization, just as the cyclophorase system is concentrated on the mitochondria and the lyases on the lysosomes. There is still much to be learned about the syntheses of proteins and nucleic acids, and about their interrelationships. The control of biosynthesis at the molecular level by feedback and other devices is an attractive field for further research. Much is now known about the chemistry of carcinogenesis, but not much of its relationship to the normal physiological mechanisms controlling growth. These are some of the more important and immediate problems.

A good deal is now known about growth at all levels, but as in other fields additions to knowledge are piecemeal, often following up clues which appear fortuitously, as in solving a jigsaw puzzle with no preview of the solution. In places, major features remain more uncertain than particular details, and any attempt to sketch in the missing parts may be very misleading. No attempt of this kind has been made here, therefore, but as far as possible the sections already assembled have been placed in their prospective places. Eventually, small further additions complete large sections of such a picture, and this era of increasing returns may be near at hand.

Nil tam difficilest quin quaerendo investigari possiet.

TERENCE: *Heautontimoroumenos*

No problem is so difficult that research cannot tackle it.

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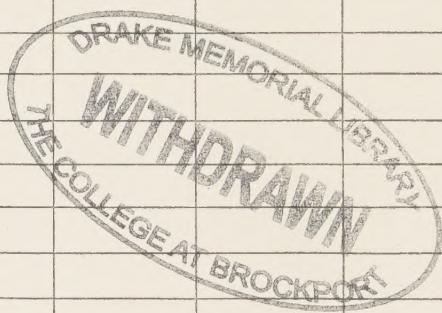
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