PRINCIPLES OF EMBRYOLOGY

BY C. H. WADDINGTON

How Animals Develop

Introduction to Modern Genetics

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WITH 186 FIGURES

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PREFACE

IN WRITING this book I have had three aims in mind. I have tried to expound a picture of embryology, which has been formed during a quarter of a century's work, and to do so in a form sufficiently factual and systematic to be useful as a textbook for students specialising in that subject, or in the allied fields of genetics or experimental zoology. At the same time, I have attempted to meet the needs of research workers in other branches of biology who wish to find out what is going on in the study of development at the present time.

Embryology grew up as a branch of comparative anatomy; and when the science is referred to without qualification, even to-day most biologists probably think first of a descriptive account of developmental changes in anatomy and histology. But there is, of course, by now a very large body of data relating to the causal analysis of development. This is often regarded as a separate corpus of knowledge, referred to not as 'embryology' but as 'experimental embryology'. A few decades ago, phylogeny and the evolutionary aspects of comparative anatomy constituted the core of animal biology, and it was not unjustified for the descriptive approach to development to be accorded the title 'embryology' *tout simple*. But now the situation seems to me to be different. The part of our subject which is of prime interest as a facet of general biology is that which deals with causal analysis, and if anyone claims to have studied embryology, this is the part which we ought to expect him to know about. I have therefore distributed the weight in this book in a manner quite different to that usual in textbooks of embryology, with more emphasis on the experimental and less on the descriptive approach. In fact, of the latter I have provided only the bare minimum which suffices to make the experimental work comprehensible. This book is, however, not intended to be for most students their first contact with embryology, but rather to serve the needs of their later university years; and it is to be expected that most users of it will have made some preliminary acquaintance with the anatomical facts, either in practical class work or through one of the many elementary texts which exist. Perhaps the ideal previous reading would be Barth's excellent *Embryology*, which has the advantage of providing not only a fuller descriptive account, but also a very stimulating introduction to the experimental analysis.

In surveying such a wide field as embryology, within a compass that can be used as a text by students, a considerable amount of selection has to be exercised. It is natural, and indeed probably desirable, that an author should devote most attention to those aspects of the subject on which he has himself worked. I am conscious that I have given more space to the

amphibia, birds and *Drosophila*, and less, say, to the echinoderms and the problems of fertilisation, than some other authors might have done. I think, however, that it is not merely a bee in my personal bonnet which has led me to include in the book a considerable discussion of topics which are conventionally counted as belonging to genetics. Embryology at the present time is in a betwixt-and-between state. It can no longer be wholly satisfied to operate in terms of the 'complex components' (such as organisers, fields and the like), which were discovered in the first successful experimental forays. On the other hand it is still too early to hope to find biochemical approaches which throw a general illumination on the scene. It is probably useful to try to formulate conceptional schemes in generalised chemical terms, such as those proposed by Weiss, or that discussed in Chapter XIX; but these must be recognised as no more than very abstract guides to possible directions which our thoughts may take. We have still to work through a region of facts and theories which deal with cellular constituents; and among this group of entities, which includes microsomes, mitochondria and such bodies, the genes (and possibly the plasmagenes) are certainly of crucial importance. It seems probable then that the most fundamental embryological theories of the immediate future will be phrased largely in terms of genes or of other bodies of a similar order of complexity; and in so far as this is true, no adequate discussion of embryology can be given without devoting a great deal of attention to the related aspects of genetics.

One of the difficulties in writing a book of this kind is to decide what references to literature should be provided. Anything approaching a complete bibliography would be too unwieldy. I have attempted two things; to provide an introduction to modern trends of work by giving a fairly large number of citations of recent papers whose results are being quoted; and to strike a balance between giving credit to the first discoverers of various facts and ideas, and indicating the most up-to-date summaries and reviews of the different topics. I can only beg the indulgence of any of my colleagues who may feel that I have either overlooked their priority or failed to recognise the soundness of a recent summing-up. In any case, the bibliographic apparatus of such a book is inevitably a forest in which the student can only too easily lose himself. I have therefore, at the end of each chapter, given a very short selection of works which are suggested as valuable further reading, either to bring the student in contact with some of the original factual material, or to introduce him to some of the stimulating ideas which run parallel to, or even contradict, those advanced in the text.

Edinburgh, July 1954 C.H.W.

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PART ONE

THE FACTS OF DEVELOPMENT

CHAPTER I

THE SCIENCE OF EMBRYOLOGY

1. *The place of embryology among the biological sciences*

The core of the science of embryology is the study of developmental phenomena in the early stages of the life-history of animals. It is, however, impossible to discover any general and important dividing line between the embryonic and later stages of development, and there is no good reason to exclude from the purview of the subject those processes of development which take place in stages later than the strictly embryonic. It is best, in fact, to understand the word 'embryology' as referring to all aspects of animal development, in which case it will include, among the peripheral fields in which it shades off into other sciences, some phenomena which may also be considered as parts of endocrinology or of genetics.

From whatever point of view one regards the biological sciences, the study of development will inevitably be found to take a central position among them. If one attempts to view biology as a whole, there are broadly speaking two main approaches which one can adopt; either one tries to formulate a general system which will exhibit all the major aspects of animal existence in their proper relation to one another; or one searches for a theory of ultimate units which could play the same role for biology as the electrons and similar particles do for physics and chemistry.

From the first, or synthetic, point of view, the most fundamental character of living things is the way in which time is involved in their existence. An animal functions from minute to minute or from hour to hour, in feeding, digesting, respiring, using its muscles, nerves, glands and so on. These processes of physiological functioning may be repeated within periods of time which are short in comparison with the lifetime of an individual animal. But there is an equally important set of processes, of a slower tempo, which require appreciable fractions of the life-history and are repeated only a few times, if at all, during one life-cycle; these constitute development. Still longer-term processes are those of heredity, which can only be realised during the passage of at least a few generations and which form the province of genetics. And finally, no full picture of an animal can be given without taking account of the still slower processes of evolution, which unfold themselves only in the course of many life-

times. From this point of view, then, embryology takes its place between physiology on the one side and genetics on the other.

As a matter of historical fact, the biological sciences at the two ends of the time-scale—those of physiology in the broad sense on the one hand, and of evolution on the other—have been more thoroughly developed than the two sciences of embryology and genetics which come between them. The volume of information available about physiological phenomena is immense; their relevance to medicine and animal husbandry has given them practical importance, and the relative ease with which they can be envisaged in physico-chemical terms has made them seem intellectually attractive. The study of evolution, which was until recently only slightly less voluminous, derived its impetus from the feeling that Darwin's work has provided the essential thread which was needed to link all aspects of biology together. Between these two huge masses of biological science, embryology and genetics are rather in the position of the neglected younger sisters in a fairy tale.

At the present time it looks rather as though the fairy tale will have the conventional ending, and the elder sisters find themselves in difficulties from which the younger ones will have to rescue them. This is becoming most apparent in connection with evolutionary studies; their enormous expansion in the past has been mainly by the essentially non-experimental methods of comparative anatomy and taxonomy, and it is already clear that little progress can be made towards an understanding of the causal mechanisms of evolution without the aid of genetics and to a lesser extent of embryology. And even physiology finds itself more and more led to the recognition that structural considerations are of the utmost importance for the functioning of biological systems; and this realisation brings it into close contact with embryology, which of all the biological sciences is most concerned with questions of structure and form.

The central position of embryology is perhaps better appreciated when one regards biology from the other viewpoint, which seeks to discover some category of ultimate units. It is clear that the unit which underlies the phenomena of evolution, and of the short-term heredity which constitutes genetics in the narrow sense, is the Mendelian factor or gene. But any theory based on our present knowledge of genes has perforce a most uncomfortable gap in it at the place where it should explain how they control the characters of the animals in which they are carried. For physiology, the basic unit is the enzyme. We know that the formation of most, if not all, enzymes is controlled by genes; in fact it is not unplausible to suggest that genes are simply a particularly powerful class of enzymes. But here once again we find ourselves confronted with that most lament-

able deficiency, our lack of knowledge of exactly what genes do and how they interact with other parts of the cell in doing it. But whatever the immediate operations of genes turn out to be, they most certainly belong to the category of developmental processes and thus belong to the province of embryology. This central problem of fundamental biology at the present time is of course being attacked from many sides, both by physiologists and biochemists and by geneticists; but it is essentially an embryological problem.

It is unlikely that the methods of classical descriptive or experimental embryology will suffice to bring any solution to the problem of the genetic control of development. Neither will the conventional breeding methods of classical genetics, or, in all probability, the normal techniques of biochemistry and physiology. A general textbook of embryology can, however, not be confined to those novel techniques of investigation which, at any given time, seem most likely to lead to major advances in understanding. New methods can usually only be applied to old material; and new ideas do not suddenly emerge full-fashioned, as Aphrodite was born from the chaotic sea; they are built up laboriously on the foundation of previous work. Thus this book will attempt to describe, in the abbreviated and simplified outline which considerations of space impose, the general framework of embryological science within which the attack on the fundamental problems has to be made. Those problems cannot always be in the forefront, but the importance of the various aspects of embryology will be better appreciated if one has a clear realisation of the nature of the goal towards which our expanding knowledge is advancing.

2. *An outline of development*

Since all animals are in some way related, through the processes of evolution, there are some similarities in their various forms of development. One can, in fact, sketch a broad outline of the early stages of development which applies, roughly at least, to all the animal phyla. This can best be described in terms of a series of stages:

Stage 1. *The maturation of the egg.* The period during which the egg-cell is formed in the ovary might be thought to come, as it were, before embryology begins, but actually it is of great importance. It is, of course, the time when the meiotic divisions of the nucleus occur and the number of chromosomes is reduced to the haploid set. Further, the egg is pumped full of nutritive materials of various kinds, collectively known as 'yolk' (in the broad sense of that word); there are usually special 'nurse cells', closely applied to the growing egg in the ovary, which are concerned in

supplying these stores of yolk. Finally, and most important of all, it is during this time that the egg-cell acquires its basic structure, which provides the framework for all the elaboration which will occur in later development. This basic structure always involves a polar difference by which one end of the egg becomes different to the opposite end; these are the so-called animal and vegetative poles. There may be also a second difference, distinguishing the dorsal from the ventral side and thus defining a plane of bilateral symmetry; perhaps indeed there is always some trace of such a difference, though it is not always well marked or very stable. Lastly one may mention a difference of another kind, between a cortex which forms the outer surface of the egg and an internal cytoplasm which is usually more fluid. We shall see that all these three elements of structure—the animal-vegetative axis, the dorso-ventral axis and the cortex-cytoplasm system—play very important roles in development.

Stage 2. *Fertilisation.* This stage involves two important processes; the union of the haploid nucleus carried by the egg with that of the sperm, and the 'activation' of the egg, which causes it to begin dividing and thus to pass into the next stage. These two processes are distinct from one another, and we shall see that activation can happen without any union of the nuclei taking place.

Stage 3. *Cleavage.* The egg-cell becomes divided into smaller and smaller parts by a process of cell division. There are many different patterns in which such cleavage can occur, and it is greatly influenced by the presence of large quantities of yolk.

Stage 4. *The blastula.* Cell division continues throughout the greater part of the embryonic period, but the stage of cleavage is said to come to an end when the next important developmental event occurs. This event is gastrulation, and the embryo which is just ready to start gastrulating is spoken of as a blastula. In its most typical form the blastula consists simply of a hollow mass of smallish cells; these have been produced from the egg by cell division, and the hollow space in the middle of the mass is formed by the secretion of some fluid material into the centre of the group. When there is a considerable quantity of yolk, the blastula becomes asymmetrical, the cells which contain a high concentration of yolk being larger than the others. In the extreme case, such as in the eggs of birds, the yolky end (the vegetative pole) does not cleave at all, and the blastula becomes reduced to a small flat plate floating on the upper pole of the yolk; this is known as a blastoderm.

Stage 5. *Gastrulation.* In a short and extremely critical period of development, the various regions of the blastula become folded and moved around in such a way as to build up an embryo which contains three more or less distinct layers (only the inner and outer layers appear in coelenterates and lower forms). These three fundamental layers are known as (i) the ectoderm, which lies outermost, and will develop into the skin and the neural tissue, (ii) the endoderm, which lies innermost and will form the gut and its appurtenances, and (iii) the mesoderm, which lies between the other two, and will form the muscles, skeleton, etc. The foldings by which these layers are brought into the correct relation with one another are very different in different groups, as they are bound to be since the blastulae from which they start may not have the typical spherical shape, particularly when there is much yolk in the egg. But in spite of differences in the process of gastrulation, the situation to which it leads—one in which there is an outer, an inner and a middle layer—is rather uniform in all groups.

Stage 6. *Formation of the basic organs.* Soon after gastrulation the fundamental pattern of the embryo begins to appear. In most cases, the organs which arise are ones which will persist throughout the remainder of development, and will form the most essential organs of the adult animal; but in some animals the embryo at first develops into a larva, forming

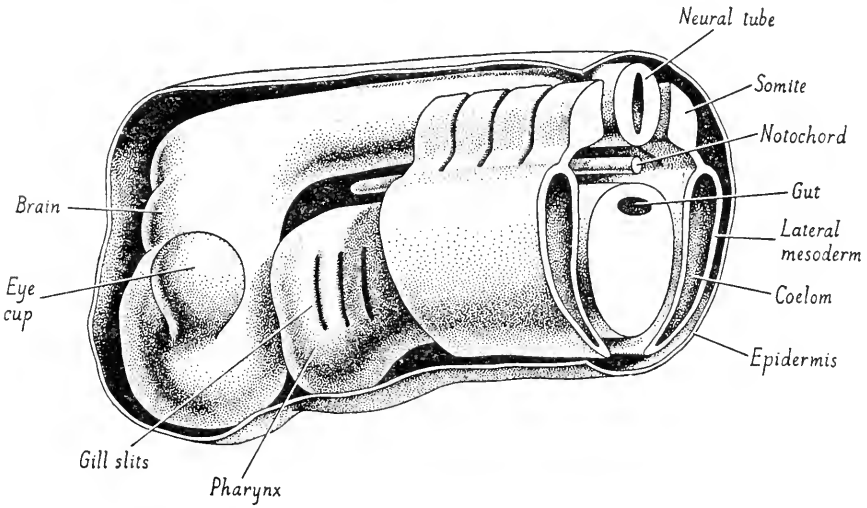

FIGURE 1.1

To illustrate the basic structure of a generalised vertebrate embryo.

organs which require radical alteration before the adult appears. There are, of course, too many types of adult or larva in the whole animal kingdom for it to be possible to give a single scheme of basic organs which can apply to them all, but it is perhaps worth while to indicate the general pattern of all the various types of vertebrates. Such a scheme is shown in Fig. 1.1. We see that the ectoderm forms, firstly, the skin which covers the whole body, and secondly a thickened plate which folds up to form first a groove and finally a tube which sinks below the surface and differentiates into the central nervous system. (At the boundary between the neural and skin parts of the ectoderm, cells leave the ectodermal sheet and move into the interior of the embryo; this 'neural crest', which forms nervous ganglia and other organs is not shown in the Figure.) The sheet of mesoderm becomes split up longitudinally into a series of zones. Under the midline of the embryo is a long rod-like structure, the notochord, which is the first skeletal element to appear. On each side of this the mesoderm is thickened and transversely segmented so that it takes the form of a series of roughly cuboidal blocks, which are known as somites, and which give rise to the main muscles of the trunk as well as the inner layers of the skin. Laterally on each side of the somites there is a zone of mesoderm which will later produce the nephroi or kidneys, and laterally again more mesoderm which is not transversely segmented and which is destined to give rise to the limbs and the more ventral muscles and sub-epidermal skin. Finally, in the most inner recesses of the embryo, the endoderm becomes folded into a tubular structure which is the beginning of the gut or intestine. The formation of these organs always begins earlier in the anterior end of the embryo than in the posterior.

After these basic elements in the adult structure have been roughed out, there remains, of course, much to be done in adding the details, but the phenomena differ so much in the various phyla that there is no point in trying to describe more stages of general application.

3. *Phylogenetic theories of embryology*

Until fairly recently, the main theoretical concern of embryologists has been to find a guiding principle which would allow them to arrange the enormous mass of descriptions of developmental changes into some sort of orderly whole. The chief such principle was found in the theory of evolution. Long before Darwin, at a time when the idea of evolution was little more than a nebulous speculation, Meckel suggested (about 1810) that a developing embryo of a 'higher' form of animal passes through a series of stages which represent the adults of the 'lower' forms ancestral to it. For instance at one stage the embryo bird has gill-slits,

structures which of course are present and have a function in adult fish but disappear in the bird before the adult stage is reached. Fairly shortly after, the improvement of microscopes made it possible for von Baer to show that an embryo never looks exactly like an adult of any kind. The gill-slits of a bird embryo are rather like those of a fish embryo, but only remotely resemble those of an adult fish.

As von Baer pointed out, the fact is that young stages of different species resemble each other more than older stages do, but this does not mean that the stages in the development of an animal repeat its evolutionary history. However, in spite of his commonsense, this idea of 'recapitulation', as it was called, was revived after Darwin had made evolution the centre of biological fashion again. Its chief exponent was Haeckel, and for some time it was taken as the guiding principle in embryology. It was sometimes argued that evolutionary change always occurs by new stages being added on at the end of development, so that the advanced animal goes through the embryonic stages of its ancestors, perhaps in an accelerated and shortened form, then goes on a step or two further. But it was eventually borne in on embryologists that von Baer had been right (cf. de Beer 1951). And as they came to reflect on the causal mechanisms underlying embryonic development, it became clear that it is only to be expected that evolutionary alterations are much more likely to affect the later stages of development, when comparatively minor features are being formed, and to leave intact the earlier steps on which all the later stages must depend. As a matter of fact, in their very earliest stages the embryos of different types of animals are rather radically different. It is at an intermediate period, early but not right at the beginning, that embryos are most alike; probably because this is the time at which the basic structure of the animal is being rapidly laid down, and it is very difficult for evolution to alter anything at such a crucial period without throwing everything into confusion.

It is, moreover, not true that an evolutionary advance always involves the addition of something new to the original course of development. In general it consists rather in a modification of the later stages in development than in an addition to them. And there are several instances in which the evolutionary novelty has been produced by arresting development at an earlier stage than previously, so that the juvenile form of the ancestor becomes the adult of the descendant. In some respects, this has probably happened in the evolution of man; the human adult has many features which remind one of the young of apes (e.g. in the large skull with the sutures between the bones closing very late, the form of the teeth, the hairlessness of the skin, etc.). It has been argued, with perhaps less plausi-

bility, that the ancestors of the whole phylum of vertebrates are to be found in the larval forms of echinoderms.

The type of analogical thinking which leads to theories that development is based on the recapitulation of ancestral stages or the like no longer seems at all convincing or even very interesting to biologists. Our interests have been awakened by the possibility of an analysis of development in causal terms; and it is in this field that modern embryology seeks for its guiding principles. Recapitulation, in all the forms in which it occurs, remains an important phenomenon, but it appears nowadays as a series of problems for evolutionary theory to discuss rather than as an explanation of developmental processes.

4. *The mechanisms of development*

During the first phases in the study of a subject, all the available resources have usually to be concentrated on the task of providing a thorough scientific description of the phenomena involved. Embryology remained in this condition until about the end of the nineteenth century, when the first serious attempts were made to investigate the causal processes by which developmental changes are brought about. The leader in this endeavour was Wilhelm Roux, who coined the title 'Entwicklungsmechanik' for such studies. This word is still commonly employed in German. Its literal translation in English is 'developmental mechanics', a phrase which is not only rather long and clumsy as the name of a branch of science, but which carries a perhaps unfortunate suggestion that only machine-like, physical processes are being envisaged. Another rather awkward phrase, 'experimental embryology', is often used in English in its place. Perhaps the most satisfactory expression would be 'epigenetics'. This is derived from the Greek word epigenesis, which Aristotle used for the theory that development is brought about through a series of causal interactions between the various parts; it also reminds one that genetic factors are among the most important determinants of development. It is, however, not yet in common use.

Since the beginning of this century, the experimental study of development has been steadily growing in importance, and is now just as indispensable a part of the science of embryology as is the purely descriptive part. Before we proceed to the detailed discussion of different types of embryos, it will be as well to give a general survey of the experimental results in as broad an outline as the summary description of the successive stages of development in the last section.

The study of epigenetic processes has been carried out by two radically different methods; those of experimental embryology proper, which

involve interference with embryos by surgical means, or by treatment with chemical or physical agents and so on; and those of developmental genetics, in which the embryos are 'experimented on' by controlling the genetic constitution of the gametes from which they arise. These two lines of approach have led to two bodies of knowledge which are as yet only somewhat imperfectly brought into relation with each other, and in the outline given below it will be convenient to treat them separately. Another point to which attention should be drawn is the fact that the attempt to understand a previously unknown causal system is nearly always a slow process. In most causal sciences, and certainly in causal embryology, a long period of investigation is necessarily devoted to discovering the general nature of the causal systems involved, and only after this endeavour has made considerable progress is it possible to get down to the concrete details of how the various mechanisms work. As will be shown below, most of the theories of experimental embryology do not attempt to do more than describe the kind of system which is operating; it is only in quite recent times, and then only in a few instances, that one can begin to envisage the specific chemical reactions or physical forces concerned.

There are three basic types of phenomena which occur during embryonic development, and for which a causal science has to attempt to find some explanation. The first is the gradual change in the nature of a mass of living matter, which may consist of a part of a cell or more usually of a group of many cells. For instance, we see the columnar epithelial cells of the early neural plate gradually assume the characteristic appearance of the central nervous system, with its elaborate arrangements of nerve fibres; or the roughly cuboidal cells of the somites become elongated and filled with myosin until they are recognisable as muscle fibres. Such phenomena may be called 'histological differentiation'; and it is most correct, indeed, to reserve the word 'differentiation' for changes of this kind even when it is used without qualification.

A second type of phenomenon is the arising of differences between the various parts of the embryo. Soon after fertilisation we may be able to recognise only two or three different regions, while at a later stage there will be many more individually characterised organs. Again within any one organ, such as the neural system, there are at first only a few distinct sub-units, in contrast to the numerous parts into which it becomes naturally divided later on (the fore-, mid-, and hind-brain, the spinal column, etc.). This phenomenon might be referred to by the expression 'regional differentiation', but actually that is usually, and better, employed to indicate the type of histological differentiation characteristic of one region (say the forebrain) when it is contrasted with that of some other

region (such as the spinal column). The arising of differences between the spatial parts of the zygote is, somewhat more commonly, spoken of as 'segregation' (or 'Sonderung' in German), words whose main drawback is that they tend to suggest a particular mechanism for the process, namely a sorting out into two separate positions of materials which were originally mingled. The word 'regionalisation' is also used as another name for the process, and is perhaps preferable, as being more neutral in its implications.

The third basic type of process is the moulding of a mass of tissue (or, in Protozoa, of a part of the cell) into a coherent structure which is recognised as having some unitary character of its own, which is usually acknowledged by giving it a name as an anatomical organ. Thus the neural plate does not merely undergo histological differentiation and regionalisation, to give separate masses of forebrain tissue, midbrain tissue and hindbrain tissue, but also becomes moulded into the characteristic shapes of these organs. The forming of a mass of cells into a new shape is known as 'morphogenesis'. In the abstract, one can conceive of it as occurring quite by itself, without any accompanying histological differentiation or regionalisation. But it is only rarely, in simple organisms such as Myxomycetes or in special situations such as cells growing in tissue culture, that this happens. Much more usually, the morphogenesis of an organ is accompanied by tissue differentiation and often by the appearance of distinct spatial sub-units (regionalisation). For such complex processes, when we wish to emphasise morphogenesis as the main component, the name 'individuation' has been proposed.

It will be realised, of course, that in the actual phenomena of embryonic development, changes of all these three types are usually closely interwoven with one another. It is true that in experimental situations histological differentiation can occur with no regionalisation and very little, if any, individuation. But regionalisation is nearly always accompanied by some individuation, since the newly appearing regions are normally related to one another in some definite pattern. And we have already noticed that morphogenesis by itself is something of a rarity. Nevertheless it is important to disentangle them from each other, since each requires a different category of explanation. Differentiation could be a purely chemical process, involving nothing more than changes in substances (which, however, might exist in larger particles than the molecules of conventional chemistry, for instance in cell granules, mitochondria, etc.). Regionalisation, on the other hand, involves some references to a spatial framework; it requires at least physico-chemical notions, such as diffusion, crystallisation or the like. Finally, the moulding of a mass of material into a shape, as in morphogenesis, can only be brought about

by the operation of forces, and thus requires discussion in terms of physics.

It is only in recent years, as our understanding has increased, that the distinction between these types of phenomena has become important for experimental embryology. The greater part of the subject has been developed in terms of more loosely defined notions, which have in practice been closer to the idea of differentiation than to the other two concepts. For instance, experimentalists have attempted to discover the factors which bring about the development of the gut from the lower end of an echinoderm egg, or that of a neural plate from a certain region of a frog's egg. Both these developments actually involve some regionalisation and individuation; but in the main the experiments have not been concerned with finding out what forces, arising from what sources, push the gut into the interior or fold the neural plate into its characteristic shape. Far more, the point has been to discover how the gut-developing region comes to differ from the parts which develop into something else, or from tissues which cannot develop at all. It is only after we have got at least some inkling of an answer to this problem of differentiation that we can proceed to tackle the other aspects of the processes which go on under our eyes. It will be more appropriate to postpone a discussion of the mechanisms of regionalisation and individuation until more of the facts have been presented (see Chapter XX), but it may be helpful to give here some indication of the general nature of the ideas which have developed concerning differentiation in the rather broad sense of that term which has just been mentioned.

Differentiation itself can be regarded as occurring in two phases. At an early stage during the development of any given region of the egg, its future fate becomes more or less fixed, so that it can only be altered within a narrow range by any known experimental means; thereafter, that region will always develop into one fairly definite end-product, provided of course that the conditions are such that it can develop at all. The process by which this fixity of end-result is brought about is spoken of as the process of determination. After it has occurred there follows a long series of events which gradually transform the cells into this adult form. These are the changes which are most usually referred to when the word differentiation is used in a rather restricted sense. During determination something occurs which decides which, out of a number of possible types of development, will actually be realised; during the later phases of differentiation, this realisation comes to pass. The most important agents controlling development are, we shall argue in detail later, the genes in the nucleus. Determination is the process of bringing into operation one or

another set of gene-activities; later differentiation is the result of these activities. Most embryological work has concentrated on the problem of determination, since it has seemed more important, and perhaps easier, to discover how the genes are brought into activity than to study the detailed course of the processes which they control. The idea of determination has therefore become one of the most fundamental in embryology. Recently, however, interest in the later stages of differentiation has been increasing, with the application of new methods, such as biochemical or immunological techniques for following the way in which specific substances increase in concentration.

The notion of determination is to some extent a relative one. It is defined in the first place experimentally, in that the part of the egg is said to be determined when we do not know any way of altering its later development, and of course it is always possible that new experimental methods will succeed where old ones fail. We can thus imagine a part being apparently determined in relation to one sort of experiment, but not yet determined in relation to some other. Moreover, there is the question of how specific is the end-result. For instance, a part may be 'determined' as eye, since it will always develop into eye whenever it can develop at all; but there may still be some possibility of controlling which part of the eye it will form (e.g. retina, tapetum, lens, etc.). Usually, in fact, a tissue gradually becomes more and more precisely determined in a series of steps as its development proceeds. Even when it has become as fully determined as it ever does, it may still have a certain restricted range of possible states which it may assume under different environmental conditions. Thus if cells from various organs of the vertebrate body are grown in tissue culture, they often lose many of their obvious visible characteristics and present an apparently 'undifferentiated' appearance (cf. Willmer 1935). They tend, in fact, to take on one or other of three basic cell forms, the fibroblastic, the epithelial or the wandering-cell types (Fig. 1.2). But when their powers of differentiation are tested by grafting them back into the body or otherwise, it is found that they are actually still as narrowly restricted as they were originally. The various alterations which the cells have undergone have not changed their essential nature, but are merely superficial reactions to different environmental conditions. They are usually known as 'modulations' (Weiss 1939). One of the most extreme examples has recently been described by Fell and Mellanby (1953); high vitamin A content in the medium causes chick embryonic skin to differentiate in tissue culture into mucus-secreting, often ciliated, epithelium instead of a squamous keratinising type. If the tissue is transferred back into a normal medium the new cells which develop are of the normal squamous kind.

In a few cases, processes which must be considered to be true 'determinations', and not mere 'modulations', may later be annulled. For instance, the parts of an early ascidian embryo have only restricted possibilities of differentiation open to them and may be considered to be highly determined; but the adult animal has considerable powers of regeneration, which demand a much greater flexibility than the embryo has at its disposal. Such phenomena are not very common in the animal world. They emphasise the fact that, strictly speaking, a given process of determination occurs only in respect of the epigenetic situation at one particular stage

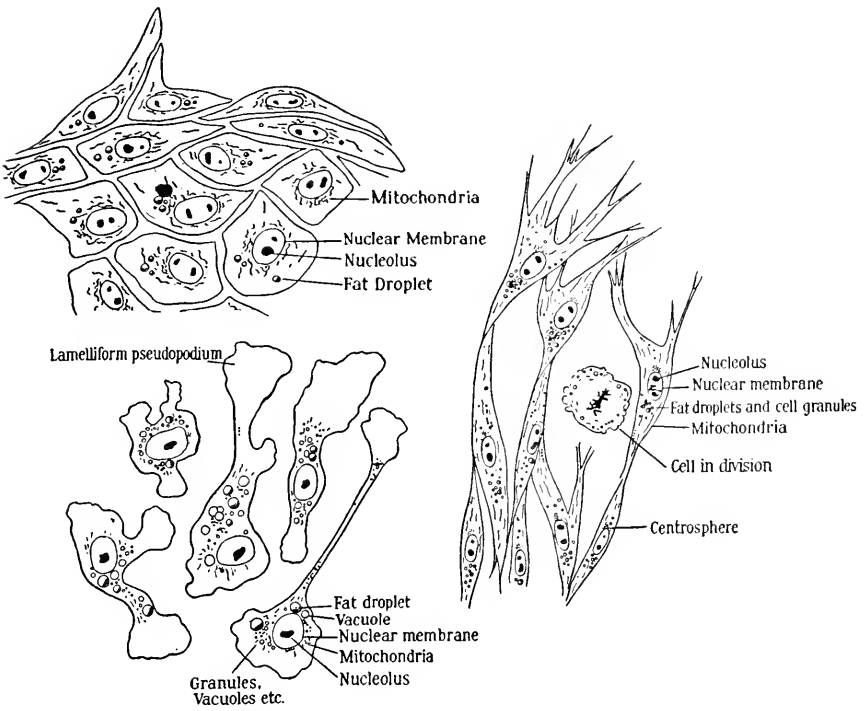

FIGURE 1.2

The three basic configurations which cells assume in tissue culture. Upper left, epithelial; lower left, wandering cells; right, fibroblasts. (From Willmer 1954.)

of development. When we say, for example, that the ectoderm of a newt neurula has been determined either to become neural tissue or to become epidermis, we mean that a choice which was open to it during gastrulation has been settled one way or the other. This need not imply anything about

choices which may become open to the material at some much later period in its history, for instance whether it will differentiate in one way or another during regeneration in the larval stage. In point of fact, it is only in a few special cases that any difficulty arises in this connection, but it is as well to bear the point in mind.

But even though the word 'determination' may, for such reasons, often need qualification to render it fully precise, the notion still remains extremely important. It enables us to deal with the fact that the fundamental causal happenings which control the course of development usually occur long before they can be visibly recognised. They are, as might be expected, chemical processes, not immediately detectable by the microscope, and at present only to be discovered by testing to see whether the developmental fate of the tissue can still be altered or not.

In very broad outline, one may say that experimental embryology has discovered three main types of mechanism which bring about determination. These are:

(1) *Ooplasmic segregation*

The different regions of the cytoplasm of the egg may have specific properties, so that a particular region can only develop in one way. Such regions are spoken of as ooplasms; an older name was 'organ-forming substances'. The actual process of development depends on the occurrence of some sort of interaction between the cytoplasm and a nucleus which will eventually arrive in the region during the course of cleavage; but it is the cytoplasm which determines the type of development. In some eggs (for instance, ascidians or spirally cleaving eggs) there may be several such substances; in others (for instance, Amphibia) there may be only one. Again the cytoplasmic regions may be precisely localised, with sharp boundaries between them, or they may shade off into one another (as in echinoderms); in the latter case, this type of mechanism grades into the 'field' type mentioned under (3). The main questions about such ooplasms are, firstly, the reasons which cause them to be segregated into different parts of the egg, and secondly, the nature of the interactions between them and the nuclei.

(2) *Evocation*

Two neighbouring parts of an egg or embryo may react with one another, in such a way as to change the capacity for development of one, or perhaps sometimes of both, of the reactants. Processes of this kind usually take place after the period of cleavage, when the shiftings and foldings of gastrulation bring together parts of the embryo which were

previously separated. By interactions between parts which have newly come together, the composition of the embryo gradually increases in complexity. Thus a region which has been determined very early, for instance, by an ooplasmic segregation, may be brought into contact with an as yet undetermined part, and exert some influence which causes that part to develop into some definite type of tissue. This type of process plays a particularly important role in vertebrates. For example, in amphibia there is an ooplasmic segregation of the so-called 'grey crescent' soon after fertilisation, which enables that region to develop into mesoderm; when during gastrulation, this future mesoderm is brought into contact with part of the ectoderm, it causes the latter to develop into neural tissue. In such cases the part which exerts a stimulus and thus causes the other reactant to develop into some tissue, say *A*, is said to 'evocate' *A*.

(3) *Field action*

In very many embryological processes, the development of any given point in a region of the egg depends on its relations with other nearby points or on its position within the region as a whole. For instance, if, at the beginning of gastrulation in the amphibia, a small piece of the mesoderm is cut out, rotated through 180° and replaced, a perfectly normal mesoderm may still be formed; the development of the rotated piece has been brought into line with its surroundings. Again, if a large part, or even half of the mesoderm is removed, the development of each point is modified in relation to its position within the total amount which is still left, so that again a normal embryo is formed. Such happenings are spoken of as 'field phenomena'. The reference of this name is to physical field theories, such as those of magnetism, gravitation and so on. The implication is not, of course, that these physical forces are operating, but merely that the biological events have the same general character as the physical ones; in both cases there must be some activity spread throughout the whole region occupied by the field, and distributed in an orderly graded manner, so that in some parts the activity is strong, in others weak, with intermediate strengths between.

In some ways, field properties are complementary to those involved in ooplasmic segregation. In the latter we are confronted with a small number of differences, usually sharply distinct from one another and each confined to a particular region; in the former with graded differences in some property which spreads throughout the whole of a wide area. From another point of view, the notion of fields is closely connected with that of evocation, since when we say that the development of one point in the

field is dependent on its relations with its neighbours, we must imply that those neighbours influence it in a way somewhat similar to that involved in evocation. In fact, one might conclude that ooplasmic segregation and evocation are the processes which occur in those aspects of development which involve sharp and clear-cut differences, such as the formation of different types of tissue, while field phenomena are found when the differences are blurred and intergrading as they are between the various parts of a single harmonious organ. This gives one hope that eventually it will be possible to see all three types of mechanism as mere variants of some more general type; but it is still too soon to attempt to do that, at any rate in an elementary discussion. (For a further discussion of embryonic fields, see the Appendix to this Chapter.)

It is probable that in every kind of egg, all these three types of process occur, although in some of them one type will predominate, in others another. Moreover, at one and the same time in embryonic development, processes of different types may be proceeding together. For instance, at the time of gastrulation in the Amphibia, the future mesoderm interacts with the ectoderm with which it is being brought into contact, and evocates neural tissue from it; but at the same time a field process is operating, by which not only is the mesoderm moulded into a full set of organs, but the newly evocated neural tissue is brought into the system too, so that a complete and harmonious embryo results. The name 'induction' is used for the whole of this complex process, of which evocation and field phenomena are separate aspects.

During the twenties and thirties the ideas sketched above were a sufficient guide to lead embryological research into ever new territories; and there are still many areas of the unknown to which they can unlock the doors. But during the last decade or so it has become increasingly clear that something further is required. The time has come to find some point of view which will suggest methods of attacking the problems of the nature of the interactions between ooplasms and nuclei, and between inducing and induced tissues or the different parts of a field. Broadly speaking, two main new approaches are being developed at the present time; one which is biochemical and cell-physiological, another which is genetical. The former is the more direct derivative of previous embryological thought. It seeks to identify and study the biochemical processes which play a crucial role in determination and differentiation, and to discover the nature and functions of intra-cellular structures which are important in this connection. Examples are the study of the physiology of organiser action (p. 193 *et seq.*), and the investigations on the biochemistry of the gradients in sea-urchin eggs and the role of the mitochondria.

Very important advances are, and will undoubtedly continue to be, made by these methods. The more strictly biochemical approach, however, encounters the difficulty that many of the happenings in a developing cell are probably related more closely to its maintenance as a living concern than to its determination and differentiation. It seems unlikely that we can hope to obtain anything like a satisfactory understanding of development in biochemical terms until we can comprehend the whole working of the cell, as regards maintenance as well as change. This consideration leads me personally to the opinion, which is by no means the most fashionable one, that it is premature to look to biochemistry to provide the main framework of ideas for embryology.

There is another approach which still requires discussion: that derived from the genetical fact that the character of differentiated organs and tissues is controlled by genes. Most people are willing to admit the relevance of this to embryology, but a study of recent books and discussion-symposia will show that in practice the contribution of genetics to embryological thought is still rather tenuous. This is in the main due to the fact that developmental genetics has been studied chiefly by people whose interests were primarily genetical, and who have posed the question: How does a given gene operate, what is the connection between a certain nucleo-protein constituent of a chromosome and some event in the cell containing it? This is obviously a fundamental question in its own right. But the progress towards an answer to it has arrived so far at little more than the statement that a change in a gene often affects the activity of a cellular enzyme or other complex molecule. From an embryological point of view, such a conclusion is somewhat trite. For embryology the question should be turned upside down; not, how does a gene operate, but how is a developing tissue affected by the genotype of the cells? We already have an answer to this which goes far enough beyond the commonplace to make a considerable difference to our whole outlook on embryological problems.

Let us therefore turn to sketch, in equally bold outline, the kind of information which has been acquired by the genetical methods of analysing development. This can be summarised as follows:

(1) There is no reason to suppose that there is any category of developmental processes which is not ultimately controlled by genes. Many of the older authors suggested that genes affect only the details of an animal's structure while the broad outlines of it were dependent on something else. This idea contained a certain germ of truth in so far as the basic plan of the animal body is laid down in the ooplasmic segregations in the fertilised egg; but we now know of cases which show that the pattern of

these segregations is itself influenced by the genes in the maternal ovary in which the egg was formed (see p. 43). The genes can therefore be regarded as the ultimate controllers of the whole range of developmental processes.

(2) It is usually held that any given gene only produces one specific immediate effect, although of course from this many secondary consequences may eventually follow in later development; and the theory is often carried a step further by the suggestion that this primary action of the gene is to influence the production of a corresponding enzyme. There is no doubt that much very beautiful work has recently revealed many genes each of which does influence the formation of a particular enzyme. But there is no very compelling reason to suppose that they do so in a single step, and that this is their primary action; nor can it be shown that all genes influence enzymes; and again it has not been demonstrated that a gene cannot have more than one primary activity, for instance by reacting with different substrates. Indeed, in the present state of our ignorance about developmental processes, it makes very little difference to our general understanding which of these many possibilities we suppose to be true. Any single gene is such a comparatively minor element in the whole complex process of the formation of a tissue or an organ that the general character of its primary action has little relevance at the present time.

(3) Genetical analysis of well-studied animals, such as *Drosophila*, has shown that each developmental process is influenced by very many genes. There must be many more ingredient elements in a developmental process than might be guessed at first sight. We cannot, for instance, hope to give a full account of the development of a nerve cell simply in terms of the synthesis of a single specific nerve protein by a system containing only one or a small number of kinds of molecules; we shall always be dealing with complex systems containing at least a few tens of different active substances.

(4) Genetic studies reinforce an important general conclusion which can also be drawn from purely embryological considerations; namely that the reactions between the many substances concerned in a developmental process are interlocked so that they become partially self-compensating. That is to say, slight changes can be made to the system without producing any effect on the end-result. For instance, most genes show some degree of dominance, which means that when one dominant allele is substituted by a recessive one, little or no difference is made to the animal which develops. Embryologically, we see the same type of phenomenon when it is found that a normal organ can be formed even if we

remove part of the tissue from which it would normally develop; or when we notice that embryonic cells usually develop either into one definite tissue (say liver) or into another (such as kidney) but not often into intermediates. The situation has been described by saying that development is 'canalised' (Waddington 1940*a*), that is, that there are only a certain number of defined channels along which the developmental processes can go; and it must be remembered that each course of development involves complex processes in which many different genes are concerned.

(5) It is obviously not the case that all genes are being equally effective in all cells of the organism; if this were so, there could be no regional differentiation. We must suppose that a group of cells follows one particular canalised process of development because one of the possible combinations of gene-controlled processes is set going, while in another group a different set of activities occurs. It is in the investigation of how this differential activation of sets of genes is brought about that the genetical and embryological viewpoints are coming closest together at the present time. We have seen that experimental embryology has developed one set of ideas about such matters; there may be a segregation of ooplasms which can react differently with the nuclei which move into them, or there may be interactions between neighbouring tissues which are sharply distinct in character (in evocation) or only quantitatively different (in field action). It is seldom, in these embryological investigations, that the genes enter explicitly into the picture, but we are dealing with the activation of different pathways of development, and these we know, on general grounds, to be ultimately under genetical control. It is, then, only a difference in the nature of the material being studied, and the techniques available, which distinguishes such work from genetical investigation into the way in which alterations in the cytoplasm or the presence or absence of certain substances in the external medium may stimulate or inhibit the operation of particular genes. This problem, is perhaps, worthy of being called the focus of present-day analytical embryology; it is discussed at some length in Chapter XVI.

There are, of course, many other principles of more or less restricted validity, which have emerged from developmental studies, but those listed above form the main body of theory which can be generally applied throughout the whole field of embryology. When one reflects on the character of these principles one realises that experimental embryology has as yet hardly reached the stage of being able to investigate the actual causal mechanisms which bring about developmental changes. For the most part, it is still concerned to discover and describe the general nature of the system which is in operation on any particular embryo. If one says

that the early development of the molluscan egg is mainly dependent on ooplasmic segregations, that does not tell us anything of the causes which bring about the segregation, or of how the various ooplasms cause the appearance of the specific characteristics of the organs to which they give rise; what we have done is to describe a type of system, but we are still unable to point to particular causes and their particular effects. The same is true when we attribute the development of the echinoderm egg to a system of gradients or fields. It is only in connection with evocation phenomena that we begin to attain any real experimental control over important developmental events, since where evocation comes into play, we can switch the development of a piece of tissue one way or another by placing it either near to or far from the source of the evocating stimulus. In this connection, then, we are already in contact with a basic causal system and can hope to go beyond finding out the general nature of the system to the crucial step of discovering what it actually is in detail. Unfortunately, as we shall see in Chapter X, it has turned out to be easier to see this bird than to put salt on its tail.

From the genetical side, also, we are as yet only just approaching the actual causal systems. We know something about the kinds of things genes, or groups of genes, do; but we still want to know exactly what some one definite gene does and how it produces its effects. It is only in a few cases that we can control the activities of genes, and it is not until we can do so, that we can hope to discover much about them. Again, evocator reactions provide one example; by the presence or absence of the stimulus we can bring into play one or another set of gene-controlled processes; but the genetic variants are not available in any of our laboratory stocks which could make it possible to analyse this situation genetically. The other instances in which it is possible to determine experimentally whether a gene shall be active or not occur in lower and more or less undifferentiated organisms (for instance in the control of immunological properties in the protozoan *Paramecium*, or the formation of adaptive enzymes in bacteria and yeasts). From such cases we cannot learn much about the precise mechanisms of differentiation, but we can find some interesting general guidance.

It is in the analysis, by developmental genetical methods, of the formation of certain particular chemical substances, that we have so far come nearest to a full understanding of any developmental process. We know a great deal, for instance, about the development of the pigments in eyes of the fly *Drosophila*; not only the genes that affect it, but also the chemical nature of some of the most important changes which those genes produce. Unfortunately, the substances about which we have such detailed know-

ledge are comparatively trivial ones; we do not have such information for anything as complex as a protein, let alone for any particular type of cell or tissue.

APPENDIX

THE CONCEPT OF EMBRYONIC FIELDS

The field concept has been widely used in some recent discussions of development, notably by authors such as Huxley and de Beer (1934),Weiss (1939) and Lehmann (1945). It is, however, rather difficult to make clear exactly what is meant by it and unless the term is given a fairly precise meaning it is only too easy to use it as a sort of 'joker' by which almost anything can be explained (see review of Huxley's and de Beer's book by Waddington 1934*b*, and Needham's discussion 1942, p. 127).

The first confusion arises from a tendency to use the work 'field' when all that is meant is a reference to the geographical location in which something is happening, while not implying anything about the nature of the events going on there. In such circumstances it is better to use a more neutral and clearly geographical term. For instance, in the neurula of an amphibian embryo the right forelimb will arise from a quite definite place. This should be referred to as the limb area, not as the limb field. At an earlier stage the localisation of the limb is not so precise. Experimentally it may be caused to appear anywhere within a somewhat larger region of the embryo. Needham has suggested that these larger regions may be referred to as 'limb districts'. We may thus speak of the 'limb district' in an earlier embryo, meaning the whole region out of which a limb could be caused to appear, and in a later stage in which the position of the limb had been more precisely fixed we could begin to speak of limb 'area'.

The word 'field' should be used only when we wish to refer to the character of the processes which go on in an area or district. By using the word we mean to imply that there are a number of processes which interact with one another in such a way that they take up definite relations to one another in space. It is easier to show what this means in a concrete example than by abstract definitions. Unfortunately there is no actual case in which the causal mechanisms of an embryological field are truly understood. It will therefore be necessary to give an imaginary example, which however will serve to show the general nature of the ideas which should be at the back of one's mind when one uses the concept of fields.

Imagine, then, a flat expanse of tissue, as it might be ectoderm on the

surface of an early embryo (Fig. 1.3). Suppose that this has the following properties: (i) that it has an anterior-posterior polarity, and that some substance *B* is present in a gradient with a high concentration at the posterior, sinking to a low level anteriorly; (2) that the micro-structure

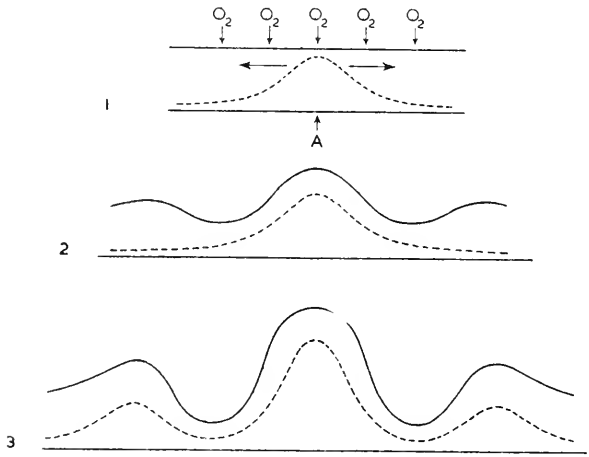

FIGURE 1.3

The development of an (imaginary) embryonic field.

(1) represents a section through a flat expanse of tissue, into which oxygen is diffusing from one side, while some substance is being formed at *A* (concentration indicated by dotted line) and diffusing outwards; (2) and (3) show sections through which the field which is developing as explained in the text.

of the tissue is such that diffusion is faster within the plane of the tissue than it is vertically through the thickness; (3) that over the whole lower surface of the tissue, there is some precursor substance *a* which can be autocatalytically converted into an active substance *A*. Now imagine that at some particular point in the region, this conversion begins to occur, and *A* to be formed (this position may be determined by the normal surroundings of the tissue, or by some experimental means). *A* will now diffuse slowly upwards through the thickness of the tissue, and more rapidly within the tissue away from its point of origin in all directions. Let us further suppose that the activity of *A* consists in causing the tissue containing it to become heaped together into a thicker layer, and also that its formation is inhibited by oxygen diffusing into the tissue from the outer surface. Then where *A* first starts to form, we shall have a heaping up of tissue, leading to a thinning of the surrounding ring of material; and in this surrounding ring oxygen will penetrate a greater relative

thickness of the material, so that the formation of *A* is reduced; while outside the ring a higher concentration of *A* will be able to build up. Thus we shall have a system consisting of a central knob of tissue surrounded by a groove outside which is a thickened ring of lower elevation than the knob. This is already a structure which could be considered as an organ, with a definite characteristic shape. We may refer to it as the organ *X*. If the substance *A* interacts with another substance *B* which is present in a graded concentration in the tissue, the shape of the organ will not be radially symmetrical round the point of origin, but will be bilaterally symmetrical.

Now it is in situations such as this that embryologists have often used the expression 'the *X* field'. They have meant two rather different things by it. The most valid use of the term refers to the situation within the region around the point at which *A* is being formed. Here we have a series of processes—of the appearance of *A*, its diffusion, its reaction with oxygen and with substance *B*, the heaping up of the tissue in one place and its thinning nearby—all of which interact on each other in a way which results in the region developing through a definite series of steps into a well-defined end-result, the organ *X*. The term 'field' is used to emphasise the co-ordinated and integrated character of the whole complex of processes. When it is used in connection with the formation of a definite organ with a characteristic individual shape, the term can be made more precise by qualifying it as an 'individuation field' (Waddington and Schmidt 1933).

The word 'field' is also sometimes used, in a rather less legitimate manner, to refer to the conditions within such a region of tissue before the point at which *A* will be formed is precisely localised. For instance, in the flank of an early amphibian embryo, the formation of a limb can be induced by implanting various substances into the mesoderm (p. 273). One may come across such a phrase as 'the forelimb field extends from about the second segment to about the tenth, reaching a maximum intensity in segments three to six'. Here we are dealing, not with the individuation field, which is confined to the area in which a limb is actually developing and the immediate neighbourhood of this, but with the large region in which there are the preconditions necessary for the appearance on the individuation field. Such a region could better be referred to as the 'region of competence' for the organ (Waddington 1934*b*) or the organ 'district' (Needham 1942). But the fact that its properties are not usually equal throughout, but are graded from a high value in the centre to low values at the periphery, has frequently tempted people to go on using the word 'field' for it; and they probably will continue to do so. Not

much harm is done by such a usage if one stops to think what one is doing.

As development proceeds, a region or district of competence gradually turns into an individuation field. One can show the character of the changes that occur in a diagram such as that of Fig. 1.4. At an early stage, any part of the district can be caused to develop into the organ by

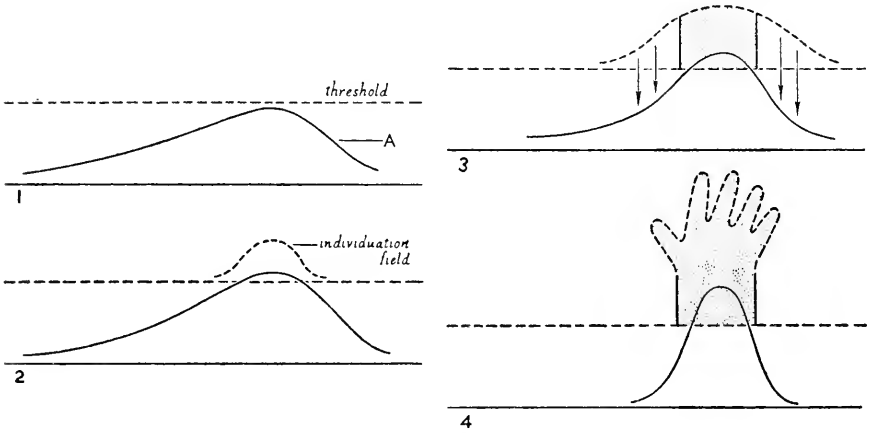

FIGURE 1.4

The development of a district of competence into an individuation field. The diagrams show a longitudinal section through a region of a developing embryo. At the earliest stage (1) the precursor (*A*) of a certain organ has not yet reached the threshold anywhere; this is the stage of a 'district of competence'. In (2) the concentration of the active substance *A* has reached the threshold and an individuation field for the organ is beginning to arise. In (3) the individuation field extends outside the area in which the organ will actually appear, which is dotted. The peripheral parts of the individuation field depress the level of *A*. This is the stage which persists in lower forms in which regeneration is possible. In (4) the individuation field has contracted until it is confined to the area of the developing organ; meanwhile the substance *A* has disappeared in the outer parts of the region, partly owing to the suppressive action of the individuation field and partly as a simple result of the progress of development.

bringing the concentration of some activity *A* above the threshold; but the ease of doing this will be distributed in a graded way. Once the threshold has been reached, and the organ begins to be formed, its individuation field gradually extends till at its maximum it covers a rather larger area than that out of which the organ is produced. In the peripheral regions, the effect of the field is to suppress any tendency for a second organ to be formed. As the formation of the organ proceeds, the extent

of the field usually contracts again until it is confined to the organ itself; and in the meantime the competence of the outlying parts of the district disappears, so that the possibility of another organ appearing lapses. In some of the lower animals, however, in which regeneration is possible throughout life, the field remains in an extended form, controlling the

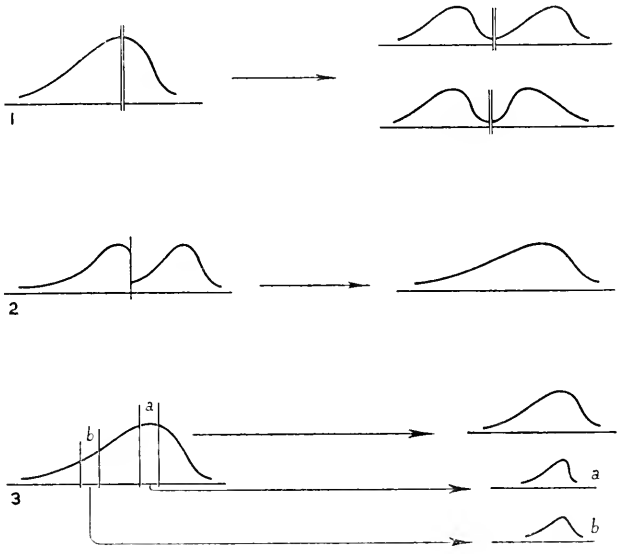

FIGURE 1.5

Behaviour of active fields.

(1) If a field is cut in half, each portion will develop into a complete unit; they may each retain their original polarity (above) or one may be the mirror image of the other (below). (2) If two fields are brought together and allowed to fuse, they form a single field (most easily if their polarity is the same). (3) If a central (*a*) or peripheral (*b*) region is removed from a field, the remainder will still form a complete unit (above), while the small isolates may each also form complete fields (below).

competence of the peripheral parts of the district and suppressing its ability to produce supernumerary organs.

When an individuation field is active, it shows many properties which remind one of the behaviour of magnetic or other physical fields (Fig. 1.5). For instance, if a field is cut in two each half may reconstitute a complete field, so that two whole organs are developed. These are often mirror images of one another. On the other hand, if two fields are brought together and allowed to fuse, they may rearrange themselves into a single

field. Again, if a part of a field, either central or peripheral, is removed, the remainder may compensate for the defect and become complete again, while the isolated part can often become modified into a small but complete field.

SUGGESTED READING

The main reference books are: for descriptive embryology of invertebrates, Heider (1936); of vertebrates, Nelsen (1953); for experimental work, Schleip (1929), Lehmann (1945, echinoderms and Amphibia only); for biochemical aspects, Needham (1931, 1942), Brachet (1944). Two of the most important forerunners of modern embryology were Driesch (see 1929) and Roux (whose views are discussed in Needham 1936*a* and Russell 1930).

There have been several general conferences and colloquia on embryology in recent years; those published by the Society for Experimental Biology ('Growth', Second Symposium, 1948), the New York Academy of Science (*Annals*, Volume 49) and in the *Revue Suisse de Zoologie*, Volume 57 (Supplement), are particularly worth reading.

For the relations between embryology and phylogeny, de Beer (1951).

CHAPTER II

THE GAMETES

DEVELOPMENT in sexually reproducing organisms is usually considered to begin at the time when the sperm unites with the egg. But actually these two types of cells are among the most complex formed in the animal body, and themselves undergo very important processes of development before they are ready to perform their characteristic functions. The undifferentiated cells which will eventually give rise to them are collectively known as gametocytes, and separately as oocytes if they will form ova, or spermatocytes if they will form sperm. The fully differentiated cells are known as gametes. The male type are called sperm, or spermatozoa, both terms being correct and with exactly the same meaning. The female type are referred to as eggs or ova, but these two words do not mean quite the same thing. The word ovum refers strictly to the gamete-cell; and this often makes up only a part of the body known as the egg, which may include a number of membranes, layers of jelly, shell, etc., which strictly speaking lie outside the ovum, and are no part of it. Thus in the hen's egg, the ovum is only that part conventionally known as the 'yolk'.

The basic functions of the gametes are, firstly, to bring together the two nuclei contributed to the offspring by the parents, and secondly to carry out the development of the new individual until it is fully enough formed to take in its own nourishment. A good deal of preparatory differentiation is required before an ordinary cell can be fitted for either task. It is not appropriate here to discuss in any detail the preparation of the gamete-nucleus, since this subject really belongs to the allied discipline of Genetics, and is fully described in textbooks of that subject. It is only necessary to remember that, whereas the nucleus of a normal body cell contains two of each kind of chromosome, and thus two of each kind of gene, in the gamete-nucleus these are reduced to one representative of each kind. The reduction takes place by a sequence of two divisions, known as the meiotic, or maturation divisions (the term 'reduction division' cannot strictly be applied to either the first or second of these, but only to both together). The matured gamete-nuclei, containing only one of each sort of chromosome, are known as haploid, while the normal condition is known as diploid.[1]

[1] In some organisms (many plants and a few animals) the body cells contain more than two of each kind of chromosome, and are then said to be 'polyploid'; in this case the gametes, if they are formed at all regularly, contain half the number in the body cells, and thus more than the haploid number; but many irregularities occur in such cases.

1. *Spermatogenesis*

In the development of sperm, the two meiotic divisions occur fairly early in the history of the spermatocyte, usually with only a short interval between them. Since there are two maturation divisions, and each division gives rise to two similar daughter cells, one spermatocyte which starts the process will eventually form four sperm. Usually they separate from one another, but in some species they remain together as a group (Fig. 2.1).

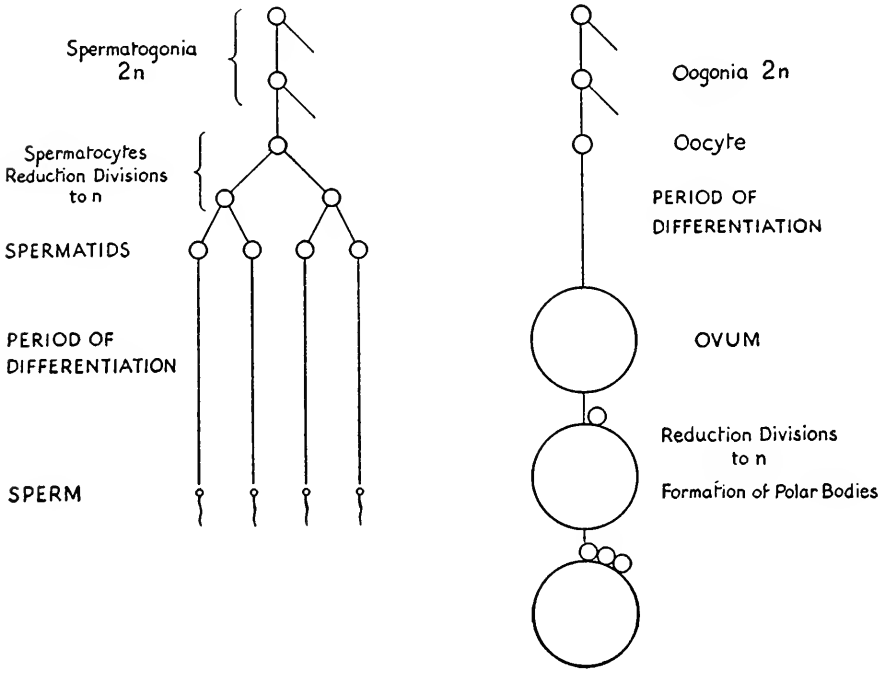

FIGURE 2.1
Diagram of the formation of sperm (on left) and eggs (on right).

At the time when the maturation divisions occur, the spermatocytes are fairly normal-looking cells; the main differentiation by which they become transformed into sperm occurs in the haploid daughter cells. In a few groups, the fully differentiated sperm are amoeboid (e.g. some Crustacea), but in most animals they are built on roughly the same plan, consisting of three main parts; a head containing the nucleus, a middle-piece containing one or more centrosomes, and a flagellar tail which serves as an organ of motion. (For a comparative account of sperm

morphology, see Retzius 1902–1909.) The processes of formation of these specialised parts of the cells have been the object of considerable microscopial study (cf. Gresson 1948) but the results have not been very clear cut, nor have different investigators always reached agreement. This is nowadays not so surprising, since studies with the electron microscope have shown that sperm contain many structures which are well below the resolving power of the light microscope, so that studies with the latter could not be expected to reveal the mode of their formation. The electron microscope work is still in its infancy, and again it is the case that agreement on the structures has not yet been reached. What is important is that this new tool has shown that the material architecture of the sperm is certainly very much more complicated than had been suspected. An indication of the degree of complexity involved may be had from Fig. 2.2, which shows the structure of the middle piece of a ram's sperm, as interpreted by Randall and Friedlaender (1950). Even if some revision later turns out to be necessary to this picture, it is impressive to discover that such an apparently simple object can contain so many structurally distinct components arranged in such definite and elaborate patterns.

The spermatozoon is a small light cell, capable of independent movement. It plays the active role in fertilisation, in contrast to the immobile and passive egg. This activity is, in fact, the basic definition of a male gamete; in some organisms the sperm is very unlike the common pattern just described, and in lower plants, for instance, there may be very little difference in shape between the female and male gametes; but wherever there is a difference in activity, we say that the more active type is the male, the less active the female. Whether there is any more fundamental similarity, other than their activity, between the male gamete of an Alga and a mammalian spermatozoa, remains rather a debatable question.

The metabolism of sperm is being very actively studied at the present time, both for its own intrinsic interest, and on account of its importance for the technique of artificial insemination. Reviews of recent work on mammalian semen and sea-urchin sperm will be found in Mann (1949, 1954) and Rothschild (1951*a*).

2. *Oogenesis*

The formation of the egg-cell is a more complicated and more lengthy process than the formation of the sperm. As a bearer of a haploid nucleus, the ovum has a somewhat simpler task than the sperm, since it does not need to produce any means of locomotion. But this simplification is more than outweighed by the fact that it is out of the cytoplasm of the ovum that the main structures of the embryo must be formed. The egg must

in the first place contain sufficient reserves of nutriment to keep the young animal alive till it can obtain its own food, and although some of these stores can, as we have mentioned, be provided outside the egg-cell proper, yet in most cases such an expedient is only resorted to after the ovum

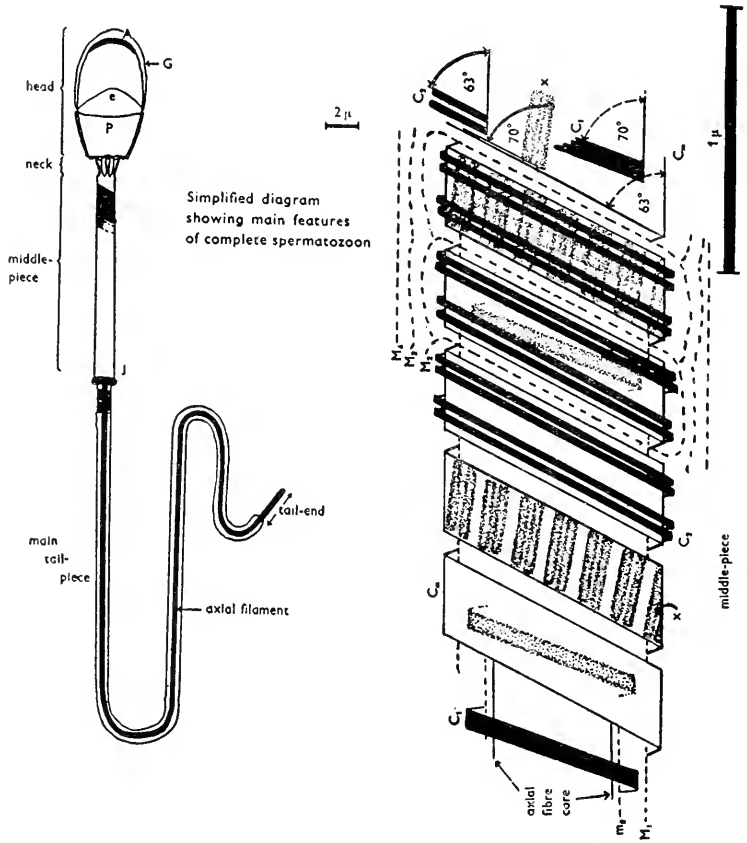

FIGURE 2.2

Structure of a typical mammalian spermatozoon (that of the ram). On the left a light microscope picture of a complete sperm, on the right an interpretation of the structure of the middle piece as revealed in the electron microscope. (From Randall and Friedlaender 1950.)

itself has been loaded to capacity. Further, apart from reserves of food, the egg cytoplasm must embody in some way the structural basis out of which the embryonic body can be formed; and this, one can see, must involve an elaborate process of preparation. It is not surprising, therefore,

that the comparatively simple maturation of the sperm is profoundly modified in the differentiation of eggs (Fig. 2.1).

The essential feature of the modification is the intercalation of a long stage of growth into the sequence. This occurs, in oocytes, before the first maturation division is completed; usually, in fact, the first division starts, and then goes as it were into a state of suspended animation while the cytoplasm and even the nucleus enlarge to many times their original volume. In some eggs (most invertebrates), the maturation divisions are not resumed until stimulated to do so by fertilisation. In others, such as most vertebrates, the egg completes its first division, but sticks again in the middle of the second until fertilised; it is only in a few types (e.g. echinoderms) that both the maturation divisions are completed before the eggs are shed from the ovary.

The intercalated growth stage not only interrupts the continuity of the maturation divisions, but also modifies the relation between the division of the nucleus and the cytoplasm. It is as if the production of one full-sized egg were as much as one oocyte can manage; if, in the middle of its process of growth, each oocyte were to divide into two and then again into four equal parts, it would have to produce enough substance to make four eggs. The necessity for such an enormous achievement is avoided by making the cytoplasmic divisions extremely asymmetrical, only a tiny lump of cytoplasm being cut off from the main body of the egg, which remains substantially intact. The first of the lumps is formed, in marine eggs, at the end of the egg which floats uppermost. This end is known as the 'animal pole' (the opposite, heavier end being the 'vegetative pole'). The small cell produced by the first maturation division is thus known as the first 'polar body'. In the second maturation division it may divide again, while a second similar small body is given off from the egg. Thus the maturation divisions, instead of producing four ova from one oocyte, finally give rise to one ovum and three polar bodies. The latter soon degenerate, and, except in very peculiar circumstances, play no part in the development of the embryo.

During the growth of the oocyte, there is considerable activity both in the nucleus and in the cytoplasm. The former enlarges greatly, becoming a so-called 'germinal vesicle', filled with a voluminous nuclear sap. It is one of the great gaps in our knowledge of oogenesis that we know so little about the constitution of this sap, except that it is rich in sulphydryl-containing proteins (Brachet 1952*a*; Brown, Callan and Leaf 1950). The chromosomes, arrested at some stage in meiotic prophase, usually tend to enlarge and become less densely staining. In highly yolky eggs which have a long growing period, this expansion of the chromosomes

proceeds very far, with the production of peculiar so-called 'lampbrush' forms in which the basic chromosome threads (the chromonemata) are clothed in a fluffy mass of thin hair-like projections (Fig. 2.3). Chromosomes at this stage stain very weakly in many of the dyes for which more normal ones show great affinity, and in particular they are difficult to stain in the Feulgen reagent which is more or less specific for desoxyribose-nucleic acid; nevertheless it appears that they never entirely lose

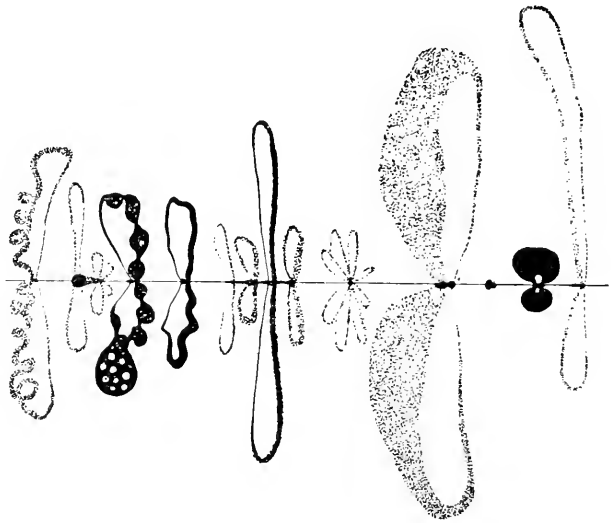

FIGURE 2.3

Structure of the loops of 'lampbrush' chromosomes from the germinal vesicle of the newt oöcyte. The chromonema runs horizontally across the drawing. It bears small swellings (chromomeres) which are double. From these arise loops, which are usually symmetrical about the axis of the chromonema, but asymmetrical along the length of the chromonema. (From Callan, unpublished.)

their stainability in this dye, and it is probable that desoxyribose-nucleic acid, which in all other circumstances appears to be an essential constituent of chromosomes, is present on them throughout oogenesis also (Callan 1952).

Accompanying the expansion of the chromosomes, there is also an enlargement of the nucleolus, which often throws off smaller bodies so that the nucleus comes to contain many nucleoli (Fig. 2.4). The chemical constitution of these is different from that of the chromosomes; they contain much ribose-nucleic acid but no desoxyribose, and also much protein of a basic type involving arginine. Substances of the same general kind as

those present in the nucleoli can be found in the cytoplasm, particularly in the immediate neighbourhood of the nuclear membrane, and it has been suggested that the nucleoli are important sites for the synthesis of basic protein-nucleates, which pass through the nuclear membrane and later control the synthesis of further cytoplasmic proteins (p. 382). The extrusion of quite large droplets of nucleolar material into the cytoplasm can

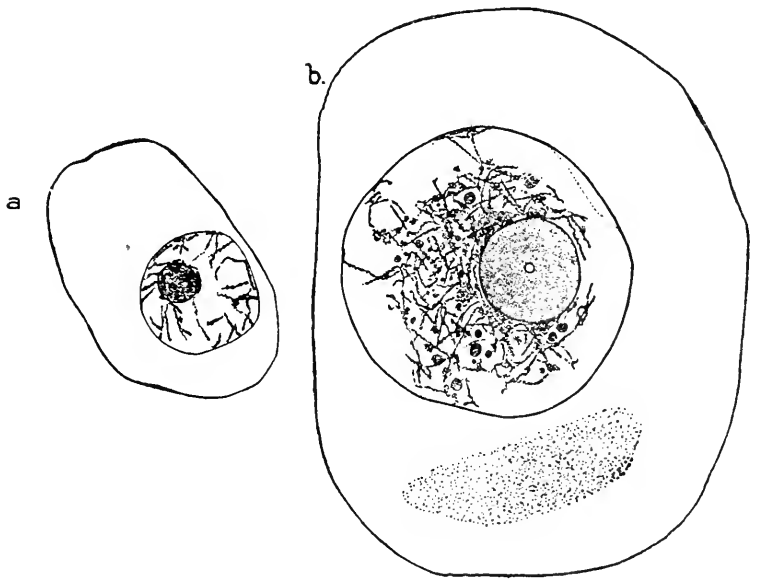

FIGURE 2.4

An early and a middle stage in the development of the germinal vesicle in the oöcyte of the snail *Helix aspersa*. The nucleus contains a large nucleolus, and by the later stage shown there are many small nucleoli in addition. The dotted region in the cytoplasm is the 'yolk nucleus'. (From Serra and Lopes 1945.)

be clearly seen in some forms (Fig. 2.5, p. 38). It is probable, indeed, that ribose-nucleic acid compounds are some of the most important constituents in the cytoplasm of the growing oocyte, since they seem to be involved in the protein synthesis which must be taking place very actively there. During the growth of the oocyte they increase in amount, but not as fast as the total volume of the cell (Osawa and Hayashi 1953). Caspersson and Schultz (1938) claimed that in *Drosophila* the quantity of such nucleotides in the oocyte was increased in eggs formed in mothers which

have extra heterochromatin (in the form of a supernumerary *Y* chromosome) but this has been disputed by Callan (1948), and it is not clear what relation exists, if any, between the cytoplasmic and nucleolar ribonucleic acid on the one hand and the heterochromatic parts of the chromosomes on the other.

In spite of the ribonucleic acid materials apparently given off from the nucleus, it is probable that the latter is not the main site of synthesis in the oocyte. In fact, Brachet (1952*b*) has argued that the nucleus contains less than its due share of the cellular enzymes, and, for instance, accounts for only a very small fraction of the respiration of the cell; this result, however, was probably a consequence of inadequate methods of cultivating the isolated amphibian germinal vesicle with which he worked, and as far as respiration is concerned, the nucleus is probably just as active as the cytoplasm (Callan 1952).

It is in the latter, however, that the major synthesis of the food reserves takes place. In many organisms there appears in the cytoplasm of the young oocyte a body which has received the name of the 'yolk nucleus'. It is particularly well seen in the eggs of some spiders, in which it has a laminated structure and is birefringent. In Amphibia it is represented in the young oocyte by a small mass in the cytoplasm which later expands and disperses to form a peripheral sheet lying just below the outer surface of the cell. It is usually considered to be constituted of mitochondria, and histochemical tests reveal the presence in it of fats, including the peculiar phosphatides which give the so-called plasmal reaction, and of several enzymes such as indophenoloxidase, dipeptidase, etc. It appears almost certain that the yolk nucleus is the seat of a particularly active synthesis of fats and proteins.

The reserve foodstuffs in eggs are often collectively referred to as 'yolk'. But this is really a loose use of the word. Strictly speaking, the true yolk is only one part of the reserve; it consists of platelets or lumps of a protein-lipoid substance. Besides it, the reserve contains globules of more or less liquid fat, and granules of carbohydrate, which are usually in the form of glycogen. In ova which contain fairly small quantities of reserves, these materials may be scattered more or less evenly throughout the cytoplasm; such eggs are known as 'oligolecithal', meaning that they contain little yolk. In most eggs with more than a very small quantity of reserve material, this is accumulated towards one end, the heavy vegetal pole referred to above; such eggs are called 'telolecithal'. There is a whole range of them, from only moderately yolky forms to bird or teleost eggs, in which the enormous mass of reserve food almost swamps the tiny patch of living cytoplasm. As a very rough general rule, the more highly

evolved animals have more yolky eggs. Thus many marine invertebrates have rather little reserve, since their embryos can at a very early stage obtain nutriment from the microscopic living creatures of the sea. Vertebrates, in which the embryo cannot feed itself until it has developed a mouth and a gut, have much more yolk; even the amphibian egg is packed with yolk platelets, most fish have still more, and reptiles and birds most of all, as well as extra-ovular reserves in the form of the 'white'. But the rule breaks down for mammals, in which the embryo is fed through the maternal placenta; although the monotremes, the most primitive representatives of the mammal stock, have eggs nearly as yolky as reptiles, in the true mammals the ovum contains scarcely any reserve. Most insect eggs, on the other hand, have a great deal of yolk, which is accumulated towards the middle instead of at one end (these are spoken of as centrolecithal eggs).

3. *Follicles and membranes*

During growth within the ovary, the cortex of the egg is usually closely invested by a layer of so-called 'follicle cells' which, presumably, play a major part in transmitting the materials for the growing oocyte; they may also be the main determinants of the cortical structure, although this is not definitely known. In mammals, the layer of follicle cells becomes very thick; in fact they increase to a largish spherical mass, within which a secretion is formed which hollows out the mass until the oocyte is hanging from a sort of stalk. This secretion contains the 'follicular hormone' which produces oestrus in the female mammal. When the egg is ripe, the follicle bursts and the egg, still surrounded by a layer of follicle cells, is set free to reach the Fallopian tubes and thus travel down to the uterus; meanwhile the remains of the follicle forms the 'yellow body' or *corpus luteum*, from which is secreted the luteal hormone, an important factor in pregnancy.

In other animals, the follicle cells are less in evidence, although probably always present. In insects the eggs are arranged in strings in the ovary; and there may be no special nutritive cells, or a group between each egg, or a single group at the end of each string with projecting strands leading down to the growing eggs (Fig. 2.5). The follicle cells or 'nurse' cells, as they are also called, are themselves often the site of active synthesis. In *Drosophila* and other insects their nuclei are polyploid, the chromosomes having divided frequently without any accompanying division of the cytoplasm (Painter and Reindorp 1939); this phenomenon is often found in secretory or synthetic cells in insects. In most cases the substances formed in the nurse-cells are passed almost completely into the

oocyte, so that the nurse-cells have almost withered away by the time the oocyte is fully grown.

The processes of oogenesis often include the production of special protective membranes to clothe the egg-cell, though a few types of marine

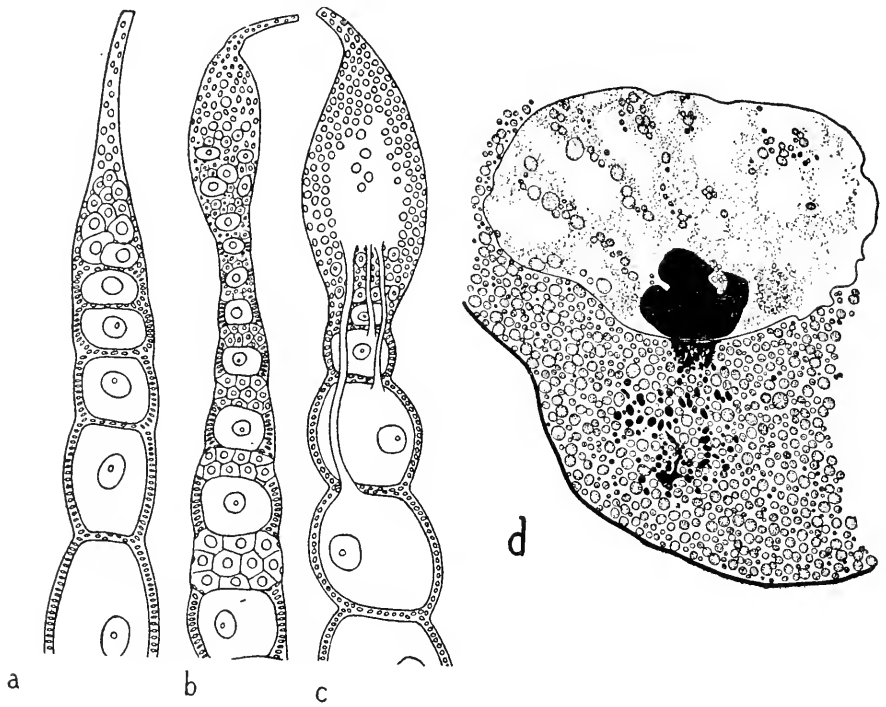

FIGURE 2.5

Figures *a*, *b*, c show various types of 'egg-strings' from the ovaries of insects. In *a* the eggs are in simple follicles (e.g. *Orthoptera*); in *b* the follicles are accompanied by nests of larger 'nurse cells' (e.g. *Coleoptera*, *Drosophila*); in *c* there is a larger group of nutritive cells at the end of the string, with channels leading to each egg (e.g. *Hemiptera*). (After Korschelt and Heider.) Figure *d* shows the discharge of material from the nucleolus of the germinal vesicle into the oöcyte cytoplasm in *Limnea*. (From Bretschneider and Raven 1951.)

eggs are shed completely naked. Egg membranes are of three kinds; those which are strictly part of the ovum itself, being secreted by its outer surface; those which are formed by follicle cells; and those which may be laid down by the oviduct during the passage of the egg away from the ovary. The first kind are usually known as the vitelline membrane, the second as the chorion, while the third may have a variety of names such

as egg capsule, or shell. Vitelline membranes are of very general occurrence, except in the few naked types of eggs. Chorions are not found quite so often; the insect egg provides a good example of them. The tertiary membranes are particularly well developed in many vertebrates (e.g. the shell and albumen of the bird's egg) but occur also in many other classes (e.g. the egg capsules of molluscs). In some animals, the membranes may be formed before fertilisation, and it is then common to find that a special opening (known as the micropyle) is provided, which allows for the passage of the spermatozoon.

The evolution of suitable egg membranes, and of eggs able to develop inside membranes, was one of the most crucial steps which had to be taken by animals in the colonisation of dry land (cf. Needham, 1931). All animals provide enough organic food reserves to keep their embryos alive until they can feed for themselves, but marine invertebrate eggs do not contain enough of either water or salts; these are absorbed by the embryo from the sea. The necessity for such salts has probably been a handicap to invertebrates in the colonisation of freshwater, and explains the relative poverty of freshwater as compared with marine invertebrate faunas. Fish go one better and provide all the salts their embryos will need, but they also do not cater for the water requirements. The same is true of most Amphibia, though in a few enough water is present in the egg to make possible a very much speeded-up development in a damp spot (e.g. the tree-frog *Hyla*). By this stage in evolution, eggs had become quite large, so as to contain enough organic matter for the development of an embryo which becomes rather complicated before being able to feed. The reptiles began the next stage, that of enclosing the egg in a shell which could include and retain suffcient water to last till feeding can start. In many of the turtles, the process is incomplete, and some moisture has to be absorbed from the wet sand in which the eggs are deposited. In birds the problem has been fully solved; and in mammals it is, of course, circumvented by the device of uterine development. It is interesting to note that water birds, instead of taking advantage of their environment to relax the effort to retain water in the egg, still provide sufficient stores for the developing embryo, and even evolve a waterproof shell to prevent more water entering. This is an example of a rather general rule of evolution, often known as Dollo's law, which states that evolutionary changes are irreversible. If a later animal returns to a set of circumstances similar to those in which one of its remote ancestors lived, it nearly always meets them, not by an exact return to the ancestral adaptation, but by some new expedient.

The evolution of an egg-shell which would retain water solved one of

the problems of land life only to raise others. The shell which encloses the water also keeps in the nitrogenous waste products of embryonic metabolism. It is a rather general rule that during the earliest stages of embryonic life the main reserve foodstuff utilised is carbohydrate (glycogen); next comes a stage when the protein is consumed, and last of all the fat (but see p. 454). It is the second of these, in particular, which gives rise to large amounts of nitrogenous waste. Animals with shelled eggs cannot avoid using such materials altogether, although they contrive to become very efficient in converting yolk protein into body protein without producing much waste; and they manage also to start rather early to consume fat, which has the added advantage that it produces some extra water as a final product of its oxidation. But even so, there is a good deal of nitrogenous waste to get rid of. In the simplest marine forms, this is excreted as ammonia, a highly poisonous but rapidly diffusible substance. In fish, more efficient excretory organs are produced, and the waste products are got rid of in the less poisonous, but less diffusible form of urea. It is characteristic of land animals, that most of their nitrogenous waste is excreted as uric acid, which is a very insoluble substance. The reason why this mechanism has been evolved is almost certainly in order to cope with the situation of the embryo within its water-retaining shell; being unable to get rid of its waste products it needs to deposit them in a form which will stay put. Thus the exigencies of ovular life have a lasting effect on the metabolism of the higher vertebrates, though mammals, which escape the closed box of an egg-shell, have returned to urea as their main excretory end-product (a fact which does not fit very well with Dollo's law mentioned above).

It is worth remarking that in animals which eventually excrete uric acid, we find that there are short early stages which excrete ammonia, like the earliest ancestors, and urea, like the rather more recent ones (Needham 1931, 1942). This is a biochemical example of the phenomenon of 'recapitulation', which we have discussed as it applies to morphological events (p. 9).

4. *The morphogenetic structure of the egg*

The nutritive materials in the ovum are more or less 'inert' in that they play little part in determining the structure of the developing embryo. As we have seen, the yolk usually occupies a definite position within the egg-cell, but it can be shifted about, for instance by centrifuging the egg, without making any great difference to the course of development. The underlying basis of the embryonic body is to be found rather in the non-yolky cytoplasm. This is often optically clear and fairly homogeneous,

and is therefore rather difficult to investigate. Many eggs contain, besides food reserves, a number of other granules, which may be grains of pigment, or mitochondria at which enzyme activity occurs, or may be of a so-far-undetermined nature (Fig. 2.6). We shall have to describe later eggs in which different regions of cytoplasm can be recognised by the different types of granule which they normally contain (e.g. in ascidians p. 106). But again, at least in many cases, the larger granules do not themselves determine the development of the different regions, since, like the yolk, they can be shifted by centrifuging without affecting it. On the other hand,

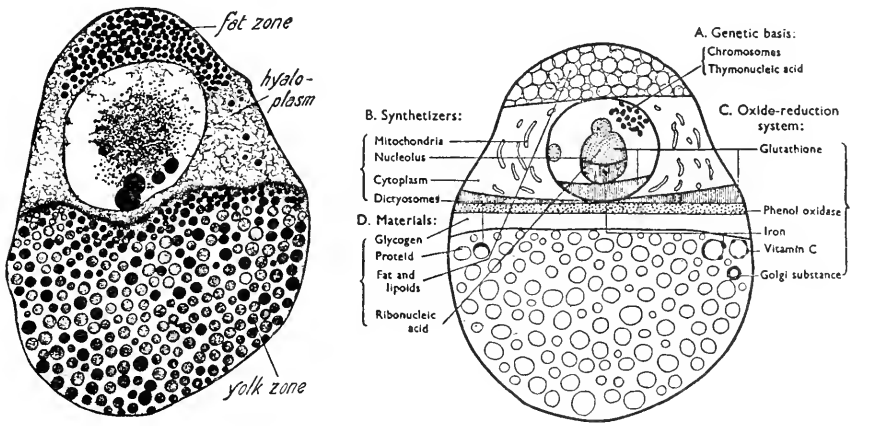

FIGURE 2.6

The internal constituents of the oöcyte of the snail *Limnea*, as seen after centrifugation. On the right the distribution of the various components is shown diagrammatically, on the left is a drawing of an actual section. (After Raven 1948, and Bretschneider and Raven 1951.)

we shall find examples (again for instance in ascidians) where, if the centrifuging moves the actual clear ground substance of the cytoplasm, together with the ultra-microscopic granules, the ensuing development is profoundly altered. In such cases, it is clear that the different parts of the egg cytoplasm are endowed with different developmental properties.

In other eggs, such as those of echinoderms, one can move even the internal cytoplasm around a good deal without interfering with development. But even in such cases, there are probably always some structures in the egg whose integrity is essential for the formation of a normal embryo. The most frequent location of this essential structure is the surface layer. The cytoplasm here is generally stiffer and more elastic than

it is within the egg-cell, forming an external layer known as the ectoplasm or cortex. It may be entirely clear, or it may contain a special layer of granules; and it may have a definite external pellicle or vitelline membrane—in any case, we shall see that a new membrane frequently forms from it at fertilisation. The cortex is so stiff that it is difficult to move by centrifuging, and, in eggs where normal development occurs after rearrangement of the interior, it seems that the reason is that the essential normal structure is still retained by this elastic external layer. We know very little about the nature of this essential structure. It is often spoken of as a 'gradient'; but this means little more than that the animal end of the egg differs from the vegetative and that there is a gradual transition between them. Probably the essential properties of the different regions depend on sub-microscopic structures in the protein framework of the cortical cytoplasm; but if this is so, we shall have to await the development of new techniques of investigation before we can learn much about them (Fig. 2.7).

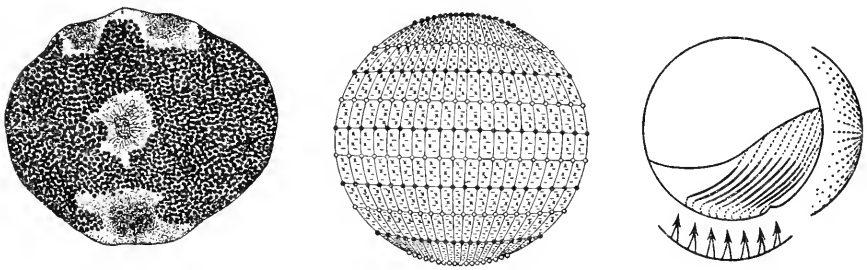

FIGURE 2.7

Three fundamentally different types of egg structure. On the left, a 'mosaic' egg with localised regions of cytoplasm (the oligochaete, *Tubifex*, after Penners). Centre, the echinoderm egg, with a general polarity, indicated by the dark and light circles, and two opposing gradients, shown by the plus and minus signs (after Harrison). Right, an amphibian egg, with a vegetative-animal gradient dependent on yolk concentration, and a cortical gradient falling off from the position of the future blastopore. (After Dalcq and Pasteels.)

In spite of the fact that our knowledge of these structural properties of the egg-cell is so slender, one must not overlook their fundamental importance. When we discuss the eggs of the different kinds of animals, we shall in every case find that the eventual origin from which the whole later development springs is the orderly arrangement of essential parts of the ovum. We must therefore enquire a little more deeply how this

arrangement is brought about. In particular, what is the relation between it and the hereditary factors or genes which determine the detailed character of the adult organism? It was for a long time argued by many biologists that genes affect only minor and trivial processes in the latter stages of development, while the major outlines of the animal body are determined not by them, but by the nature and structure of the egg cytoplasm. This theory made no suggestion as to what determined the nature of the egg in its turn, and was therefore always somewhat incomplete. But this was not its worst fault; in the only suitable cases which have been properly investigated, we have evidence that the theory is incorrect.

There are not many examples of animals in which there are two or more variants in the basic structure of the egg cytoplasm, but a few cases are known. For instance, the eggs of molluscs cleave with a spiral pattern (p. 60); and this spiral may be either right- or left-handed, a few species having variants of the two kinds. In the pond-snail *Limnea*, the inheritance of such variations was studied by Boycott and Diver (1923, 1930). The right-handedness or left-handedness of an egg depended in a straightforward way on the genes in the mother in whose ovary it was formed. This is a clear case of a difference in egg cytoplasm, which is inherited by means of genes, exactly as are differences, for instance, in the formation of pigment in an eye-cell. The only slightly odd feature of the situation is that, whereas one normally looks for pigment within the cell in question, we find it more convenient to diagnose the nature of *Limnea* egg cytoplasm by waiting to see which way the fertilised egg will cleave; but that is a mere matter of the technique of study. The important point is that in this case we can prove that the fundamental outline of the embryonic organism is based on the egg cytoplasm, but that this in its turn is determined by genes, just as any other character is (Fig. 2.8). It is still quite uncertain how these genes operate. One possibility is that they influence some asymmetry which might exist in the protein molecules of the egg cytoplasm; but this is rendered unlikely, though not perhaps impossible, by the fact that the structure of the sperm, which are also spiralised, is not affected by the genes which control the symmetry of the eggs (Selman and Waddington 1952).

Other examples of the control of the egg cytoplasm by the genes in the maternal ovary are provided by the 'female-steriles' in *Drosophila* (p. 135).

We may almost certainly conclude that in all eggs the basic structure of the cytoplasm is laid down in the maternal ovary while the ovum is being formed, and that it is as fully dependent on the genes of the mother as is the character of her eyes or skin or hair. If this is so, we should expect to find a regular relationship between the main structure of the egg and

the position in which it lies in the ovary. This question has not had as much attention in recent times as it deserves. It is known that in many insects with elongated, banana-shaped eggs, the long axis of the egg always lies along the long axis of the insect; in some invertebrates, the position of the egg nucleus and of the accumulation of yolk has a definite relation to the blood supply, and the same has been claimed to be true of frogs' eggs. But in most of the types of eggs in which the internal structure is clearest (e.g. ascidians) we know very little about the geometry of their formation within the ovary.

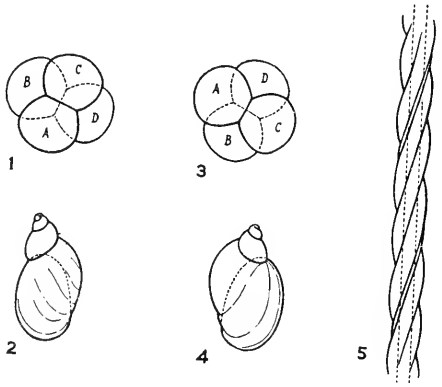

FIGURE 2.8

The 4-cell stage and the adult shell of dextral (*1*, *2*) and sinistral (*3*, *4*) *Limnea peregra*. (After Robertson 1953.) (5) Shows part of the tail of *Limnea* sperm, consisting of three large and one small strand wound, always in a dextral spiral, round a central core. (After Selman and Waddington 1952.)

SUGGESTED READING

Rothschild 1951*a*.

CHAPTER III

FERTILISATION

THE MOMENT of fertilisation is the conventional point of origin from which to date the existence of a new individual. We have seen in the last chapter that in fact many processes which are most important for the developing embryo occur before fertilisation, during the maturation of the egg. It cannot be denied, however, that fertilisation is, normally at least, the most crucial event within the continuous series of changes by which the new creature comes into being. It is not a simple occurrence, at which there is only one happening of importance; but its two important phases succeed one another quite quickly, and although they can be dissociated from one another in experiments, they are normally closely bound up with each other, so that fertilisation appears as a single, though complex, event (Fig. 3.1).

It would be out of place to discuss here the many and various mechan-

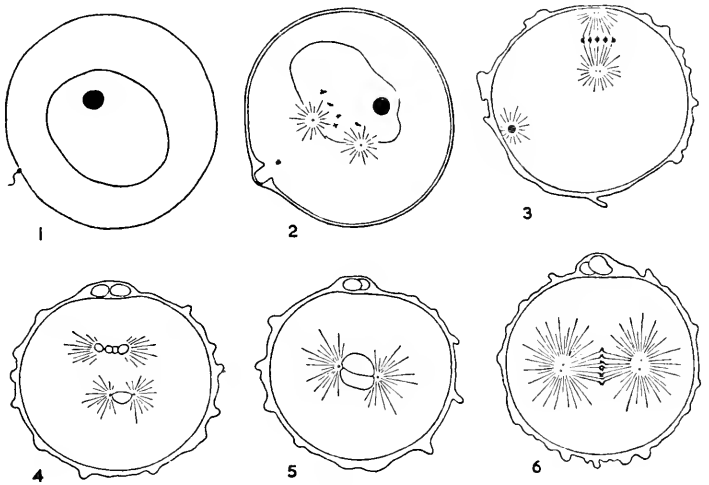

FIGURE 3.1

Fertilisation in the annelid *Urechis*. In *1* a sperm is entering the egg at the bottom left. In *2* the egg has formed a fertilisation cone and the germinal vesicle is breaking down; *3*, second polar body division and sperm aster; *4*, egg and sperm nuclei approaching one another—polar bodies at animal pole; *5*, union (conjugation) of male and female nuclei; 6, first cleavage division. (After Belar.)

isms which have been evolved to assist the male and female in bringing their gametes into proximity; for details of copulatory and other devices, reference must be made to works on the general biology of sex. We may take up the tale of fertilisation from the point when eggs and sperm are in each other's presence. The first reactions between them are, in many cases, chemical. It was shown by Lillie in 1913 that the water in which eggs of sea-urchins had been lying is able to induce a reaction in sperm of the same species, which are caused to agglutinate together into clumps. This is due to a substance given off by the eggs, to which the name 'fertilizin' was given. It has since turned out that this substance is none other than the jelly (or some constituent of it) by which the eggs are surrounded. It reacts with a substance in the sperm, which has been named 'anti-fertilizin'. Very little is known about the nature of these substances, except that they are both proteins; the reaction between them is probably similar to that between antibodies and antigens. Such substances have only been definitely proved in some of the marine invertebrates, but they may perhaps be present in all animals, since the agglutination reaction by which they are recognised is really due to an excessive performance of their real task; a fertilizin which reacted with sperm, but did not go so far as to immobilise it by agglutination would easily escape detection.

It has also been claimed that sea-urchin eggs secrete a substance which attracts sperm, so that the latter move by chemotaxis towards the eggs. There seems, however, to be no conclusive evidence of this, and opinion seems to be crystallising against it. On the other hand, there do appear to be sperm secretions which affect the egg, assisting the sperm in the task of penetrating the egg surface or the jelly which surrounds it. (In mammals, enzymes having this function occur in the secretions of glands which contribute to the semen, and it is not clear that the spermatozoa themselves produce anything of the kind.)

All active substances produced by either eggs or sperm and acting on gametes of the opposite sort are sometimes collectively known as Gamones, the egg secretions being Gynogamones and the sperm secretions Androgamones. In this terminology, fertilizin becomes Gynogamone II and anti-fertilizin Androgamone II. The subject has recently been reviewed by Tyler (1948, 1949), Runnström (1949) and Rothschild (1951*a*, *b*), and further details may be found in their papers.

The actual process of fertilisation starts when the sperm first touches the egg surface. As mentioned above, the ensuing processes fall into two phases. These are:

(i) The activation of the egg.
(ii) The union of the two haploid nuclei.

(1) *Activation*

By 'activation' we mean the setting in train of a series of changes which bring the egg out of the quiescent state in which it awaited the arrival of a spermatozoan and start it off on the course of development. These changes take somewhat different forms in different groups, but there are certain common elements which are nearly always found. First in point of time, there may occur, in eggs surrounded by thick jelly, a reaction of the egg surface which assists the sperm in penetrating these outer coverings. For instance, in some echinoderm eggs, a conical projection pushes out from the egg surface and, as it were, catches hold of a sperm and draws it inwards through the jelly. Such happenings are, however, not found in all eggs.

Some kind of surface reaction of the egg is nearly always produced by the sperm. The most important and widespread form of the reaction is a change by which the first sperm which penetrates renders the egg surface impenetrable to later sperm. The exact nature of this change is still unknown; it may even differ in different groups. In many of the naked marine eggs, it is made visible by the formation at the surface of the egg of a new membrane, the 'fertilisation membrane', which lifts a little way off the egg immediately after activation (Runnström, 1952*a*, *b*). This is very well seen in echinoderms; and in them it appears to be formed by the swelling and breaking up of a thin layer of colourless granules which can be found just below the surface of the ripe, unfertilised egg (Fig. 3.2). It seems, however, that in some species of echinoderms if not in all, the

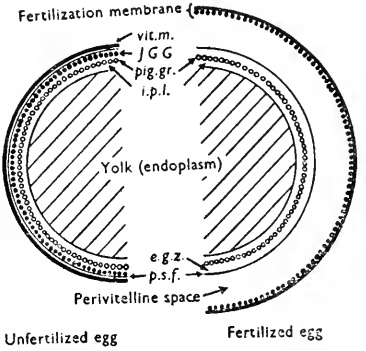

FIGURE 3.2

Diagram showing the elevation of the fertilisation membrane in the sea-urchin egg. In the unfertilised egg there is an inner layer of pigment granules (*pig. gr.*) and an outer layer of cortical granules ('Janus Green granules', *JGG*). The perivitelline space appears between these two layers. (From Runnström 1952, after Motomura.)

impact of the first spermatozoan causes a cortical change in the egg which spreads over the whole surface in a much shorter time than it takes for the fertilisation membrane to appear. Rothschild and Swann (1949) were able to reveal this change by the use of dark ground illumination, and they present some reasons for thinking that it is this almost immediate effect, rather than the relatively slow elevation of the fertilisation membrane, which constitutes the block to polyspermy.

A surface change which guards the egg from the entry of more than one sperm can, however, occur without any visible sign of a fertilisation membrane. In fact, a change of this kind seems to be a quite general part of the activation process, excepting only in some of the very large, extremely yolky eggs, such as those of reptiles and birds and some insects. In these, the entry of considerably more than one sperm is a normal occurrence; only one sperm nucleus fuses with the egg nucleus, and the remainder gradually disappear after remaining for a time in the region where the cytoplasm mingles with the yolk, in the digestion and assimilation of which they may play a part (and see p. 62).

Changes of the egg surface are not always the only visible signs of activation. In many eggs, the penetration of the sperm initiates a more or less complete rearrangement of the internal constituents (Fig. 3.3). Other examples are described in detail later (see ascidians, p. 106, Amphibia p. 146). In these cases the pattern of the egg before activation bears little obvious relation to that of the embryo which will develop from it, while after activation a clear connection can be traced; thus we may say that in these forms, activation is the final stage in preparing the egg for the series of foldings and bendings by which the embryonic body will be shaped. These internal results of activation can only be discovered at all easily if there are differences in colour or texture between the various regions of the egg which make it possible to follow their movements after the sperm penetrates. It is therefore only in certain favourable types of eggs that they have been described, and it is still somewhat uncertain how generally they occur. The evidence suggests that there may always be some internal rearrangement, but that it is often quite small in extent.

One aspect of activation which may be specially mentioned is the determination of the plane of bilateral symmetry. Most eggs, as has been stated, have before fertilisation an axis of symmetry running from the animal pole (where the polar bodies are formed) to the vegetative (yolky) pole. Some eggs (e.g. of insects) are bilaterally symmetrical, but in most types there is no sign in the unfertilised egg of anything corresponding to a 'Greenwich meridian'; and it may turn out in later development that the plane of bilateral symmetry is related to the point of entry of the sperm.

Considerable controversy has raged about whether the entry of the sperm fixes the position of the dorso-ventral plane, or whether this is already determined in the egg and some mechanism ensures that the sperm always enters on it. The evidence is still somewhat conflicting, but seems to indicate that there is something in both ideas. It is certainly true that if eggs (e.g. of frogs) are artificially fertilised by sperm placed on the surface, the point of entry of the sperm determines the plane of bilateral symmetry;

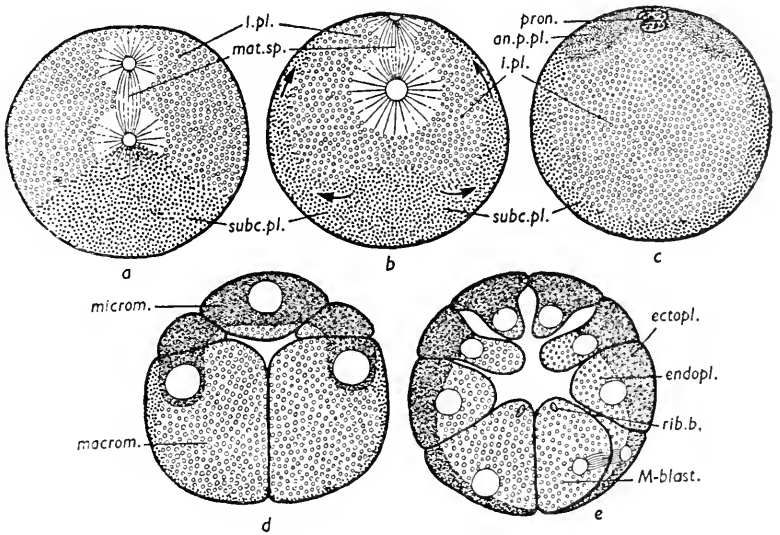

FIGURE 3.3.

Movements of oöplasms following fertilisation in *Limnea*. (*a*) Shows the vegetative plasm (dots) before fertilisation. The entry of the sperm is followed (*b*) by a movement of this plasm towards the animal pole. A little later (*c*) the nuclei (*pron.*) conjugate near the animal pole and a new oöplasm (close dots) appears there. During the cleavage divisions, this extends towards the vegetative pole (*d* and *e*), the original vegetative plasm becoming less easily recognisable. (After Raven 1948.)

but it is also probable that there is a predisposition of a fixed plane in the egg, which controls the point of entry in normal unforced fertilisation. (For a full account of the rather complicated events following fertilisation in frogs, see Ancel and Vintemberger 1948 and p. 146.)

Accompanying the visible structural changes produced by fertilisation, there are almost certainly associated alterations in the biochemical processes proceeding in the egg. Many years ago Warburg showed that the oxygen uptake of fertilised sea-urchin eggs is very considerably higher

than that of unfertilised eggs, and it was thought that fertilisation brought about a great increase in respiration. However, more recent work has shown that the difference he described is due rather to a decrease in oxygen uptake by the stale unfertilised eggs than to an increase in the fertilised ones. Rothschild (1951*b*), in a recent discussion of the metabolic changes produced by fertilisation, is very cautious about the extent of our knowledge on the subject. He suggests that the production of an acid of unknown nature is one of the most certain and striking phenomena, and lists a number of other changes, such as a reduction in glycogen content and a fall in respiratory quotient, without feeling justified in deciding which if any of these are of major importance.

(2) *The union of the nuclei*

The union of the two haploid nuclei of the egg and sperm is, from the long-term point of view, the most important phase of fertilisation. It is an essential part of the system of reproducing diploid adults by means of haploid gametes which has proved itself most efficient as an evolutionary mechanism and has therefore been perpetuated in the vast majority of animals and plants. The genetical and evolutionary aspects of the phenomenon fall outside our immediate field of interest. We must, however, give some account of the actual process by which the two nuclei come together.

The essential parts of the sperm in this connection are the head, which contains the nucleus, and the middle-piece, which contains a centrosome or spindle-body. The tail is primarily a locomotor organ, and its functions are finished when the sperm head becomes attached to the surface of the egg. It is often discarded at this point, only the head and middle-piece penetrating; and in those cases in which the tail goes in too (e.g. in mammals), it soon degenerates and plays no known part in later events.

The actual coming together of the two nuclei is simple, though mysterious enough if one tries to imagine how it works. The sperm nucleus after penetration always moves off towards the egg nucleus, while the latter sometimes moves to meet it; what moves them, and how the movements take the correct directions, are quite unknown. The sperm head (or as one may now call it the male nucleus, often called the male pronucleus), is accompanied by the middle-piece or centrosome. This body has a strong tendency to cause the cytoplasm around it to form an 'aster', which is a spherical aggregation of radiating fibres. If often starts to do so soon after getting inside the egg; but it soon divides, and an aster begins to form round each of the daughter centromeres. Where these two asters come together, a still more strongly fibrous body is

formed, known, because of its shape, as the 'spindle'. This is the first cleavage spindle of the egg, and provides the mechanism for the first division of the chromosomes. It will be seen that the egg centrosome normally takes no part in it, though there are certain exceptions to this rule.

Meanwhile, changes have been taking place in the male pronucleus. The originally compact sperm head swells and takes on a normal nuclear appearance; probably this swelling, which must involve the imbibition of water from the cytoplasm, is largely responsible for the formation of the sperm aster, which is much more highly developed than such structures are in the later cleavages. The two pronuclei, as has been said, move together, often to some rather definite position in the egg. They rarely fuse entirely before the nuclear membranes break down and the chromosomes arrange themselves on the metaphase plate of the first cleavage spindle. It is, in fact, only at the first cleavage that the essential union of the two haploid nuclei is finally consummated, and fertilisation can finally be said to be complete.

The two aspects of fertilisation, which we have distinguished as activation and the nuclear events, are not in fact completely separate from one another, but have certain interactions. An interesting example of this has been described by Allen (1954). He sucked an echinoderm egg into a narrow tube, so that it became considerably elongated. If, in such an egg in which the nucleus is located at one end, the fertilising sperm is introduced at the other end, only this latter end becomes activated; that is, it is only at this end that the cortical granules break down and the fertilisation membranes form. From a variety of experiments of this kind, the conclusion could be drawn that the nucleus tends to inhibit the breakdown of the cortical granules in its neighbourhood. At the same time, the events in the cortex have a reciprocal influence on the nucleus. If the germinal vesicle lies in a region in which the cortical granules remain intact, it seems unable to migrate towards the sperm nucleus, and does not divide, although its nuclear membrane disappears at the same time as that of the sperm nucleus.

3. *Artificial parthenogenesis*

The activation effect of the sperm can be separated in experiment from its action in bringing the gamete nuclei together. Thus Hertwig, in 1916, showed that sperm are still effective as activating agents after they have been given a dose of X-rays which entirely puts out of action their nuclear component. The sperm nucleus can also be rendered inviable by other means; for instance by ultra-violet or by certain chemicals such as trypaflavines. Eggs fertilised by such sperm can develop normally, but they will

contain only the maternal chromosomes and hereditary factors. If the sperm used belonged to a different species to the egg, apparent hybrids appear which, however, have only maternal characteristics. They are known as gynogenetic hybrids. They often survive better than true hybrids between the two species concerned, since their development is not complicated by the presence of the paternal genes which may be incompatible with the egg cytoplasm.

It is possible to go further than this, and eliminate not merely the sperm nucleus but the sperm as a whole. Several authors in the eighteen-nineties (Morgan, Hertwig, Loeb) found that ripe eggs of various species could be activated and started on a course of development by purely chemical or physical treatments. In the early years of this century a great deal of work was done on the subject and many different treatments were worked out for different types of eggs. A very large number of agents were found to be effective. For instance temperature shocks, both hot and cold, the action of acids, changes in osmotic pressure, ultra-violet irradiation, physical puncture with the tip of a needle, etc. No one procedure works satisfactorily over the whole range of animal species.

The development of an egg without fertilisation is known as parthenogenesis. The procedures for artificial parthenogenesis have in the first place been worked out empirically, as a series of 'cookery book recipes' which experience has shown to be effective in the particular species of animal being studied. There have, of course, been many attempts to formulate a theory which will account satisfactorily for the effectiveness of the various agents. The most important of these are the following:

(1) Loeb argued that the most effective procedures involve two stages of treatment. He suggested that the first step is to produce a superficial cytolysis of the egg cortex, which he thought was associated with an increase in respiration. In many species this step can be produced by the action of acids or a temperature shock. The second step is to apply a protective treatment which prevents the cytolysis going too far. This may often be done by treatment with a hypotonic solution. The difficulty with this theory is that the notion of cytolysis is so ill defined as to have little definite meaning.

(2) F. R. Lillie ascribed the main role to the operation of fertilizin produced by the egg itself.

(3) Dalcq, Heilbrunn and Pasteels, emphasised the importance of calcium in the medium.

(4) R. S. Lillie considers that the parthenogenesis is brought about by an activating agent (*A*) which is produced from two other substances, a

product of hydrolysis (*B*) whose formation is stimulated by acid, and a synthesised substance (*S*), the concentration of which is increased by hypotonicity.

(5) Bataillon, like Loeb, emphasised the double nature of the process. In his view one element is the production of a cortical change, and the second the production of the cleavage apparatus in the interior of the egg.

Recent discussions of the pros and cons of these various theories may be found in Dalcq (1928), Tyler (1941) and Brachet (1944). The fact that there are so many and such different theories still in the field indicates that none of them is very satisfactory. The process of activation must almost certainly be complex and involve at least the two factors emphasised by Bataillon; that is to say, an action on the cortex and an action on the internal cytoplasm. Little more need be said at present about the cortical effect than has been given above in the discussion of normal fertilisation. It may be mentioned, however, that it is not uncommon for parthenogenetically activated eggs to fail to produce a plane of bilateral symmetry, but to develop into radially symmetrical forms. This is another demonstration that, in the species in which this happens, the point of entry of the sperm plays an essential role in determining the dorso-ventral plane. The failure of this plane to appear in parthenogenesis is presumably a consequence of the fact that the activating agent in this case operates simultaneously over the whole surface of the egg.

Several very interesting facts have emerged in recent years about the internal cytoplasmic events in parthenogenetically activated eggs. In the normal development of almost all types of animal eggs the spindles for the cleavage divisions arise from centrosomes which have been brought in by the sperm. The centrosome remaining in the egg from the last maturation division normally degenerates and takes no part in the later cleavages. We have to inquire, therefore, whence the centrosomes for the cleavage spindles come in cases of parthenogenesis when no sperm centrosome is available.

In some forms there is no doubt that the spindles which arise in connection with the formation of the polar bodies can in these circumstances take over the control of the cleavage division. A case which has been studied in detail is that of the echiuroid worm *Urechis* (Tyler 1941, Fig. 3.4). The egg of this form when laid contains a large germinal vesicle, and at the animal pole there is a deep indentation of the surface. Treatments either with hypotonic solutions or with ammoniacal sea-water can bring about activation, which is normally made visible by the rounding-up of

the egg and the elevation of the fertilisation membrane. After short exposures to activating agents these two changes are delayed and there is no sign of any extrusion of polar bodies. Eventually, however, the egg rounds up, the membrane rises and the egg proceeds to cleave, the cleavage spindle being, in fact, that which would normally have given rise to the first polar body. Slightly longer exposure results in what appears to be normal activation. The egg rounds up, the membrane rises and the two polar bodies are formed in normal sequence. The eggs, however, then usually fail to undergo any further cleavage, apparently because they

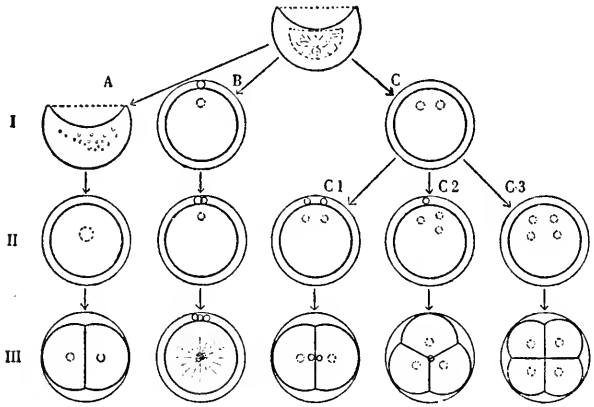

FIGURE 3.4

Parthenogenesis in the echiuroid worm *Urechis*. The upper figure shows the unfertilised egg, in which the animal pole is depressed. Columns *A*, *B* and *C* represent the behaviour following increasing exposures to ammoniacal seawater. Row I, time of first polar body formation; row II, time of second polar body formation; row III, time of first cleavage. (From Tyler 1941.)

contain only a single aster and not a bipolar spindle. With still longer exposure the rounding up of the egg and the membrane elevation proceed normally, but the first polar body division takes place inside the cell, which is thus provided with two nuclei at each of which a spindle appears ready for the second polar body division. At this second division two, one, or no polar bodies may be extruded, leaving two, three or four nuclei still inside the egg. The eggs then divide into the corresponding number of cells and continue their development to give normal larvae. It is clear in this case that although in some ways the activation is most nearly normal with that treatment which allows the polar bodies to be formed in the

usual manner, yet this gives the worst results in later development, because the eggs are left without a proper spindle mechanism to control the cleavages.

In other types of parthenogenesis new cleavage spindles may arise independently of the polar body spindles. For instance, the most effective treatment for the parthenogenesis of frogs' eggs consists of pricking the egg with a sharp needle. But this is effective only if the needle carries into the egg some foreign protein. Normally sufficient such material is present in the form of the cellular debris adhering to egg jelly. It was originally thought, e.g. by Bataillon, that it was necessary to inject a complete nucleated cell. Recent studies by Shaver (1953), however, have shown that the effect is actually brought about by ribo-nucleo-protein granules. He made the interesting observation that the granules of this kind present in the unfertilised egg are without effect, but they rapidly acquire effectiveness just at the time when the blastula is developing into the gastrula. This is interpreted by Brachet (1952*a*) as another indication that the synthesis of new proteins begins to occur in the embryo at that time. The action of these foreign proteins on the egg cytoplasm has not been followed in detail, but there is no doubt that their main effect is to cause an 'aster' or cleavage spindle to arise, possibly by some action rather like that of coagulation.

In some cases cleavage spindles appear to arise quite spontaneously without any connection with polar body spindles or with introduced foreign proteins. For instance, the eggs of some echinoderms can be broken by strong centrifugation into a number of fragments of different specific gravity. Only one type of fragment contains a nucleus; the others are completely non-nucleated. They can, however, respond to activation treatments similar to those which are effective on normal eggs. Asters then appear in the cytoplasm and the fragments become divided up into smaller lumps of cytoplasm which can apparently be regarded as cells, except that they do not contain any nucleus (Harvey 1936, 1940*b*). This absence of the nucleus is presumably responsible for the fact that the cleavage figures remain as single asters and do not unite in pairs to form spindles. Nevertheless the 'cleavages' to which they give rise continue for a considerable time and occur with some regularity. It seems that the centrosomes around which the asters are organised are fully normal, and are therefore endowed with the property of genetic continuity. As Tyler (1941) has pointed out, this means that bodies endowed with the capacities for identical self-duplication and division can arise spontaneously in the cytoplasm. It might be said, indeed, that we have here an instance of the appearance of new plasmagenes (cf. Chapter XVIII).

A final point of interest in connection with parthenogenesis concerns the number of chromosomes found in the resulting embryos. The treatments which bring about artificial parthenogenesis do not usually cause a complete suppression of the maturation divisions, which would produce diploid eggs, such as those characteristic of the diploid parthenogenesis which is a normal means of reproduction of many species in nature. On the contrary, the activated egg as a rule contains a haploid nucleus or nuclei, and might be expected to develop into a haploid individual. This is indeed what very often happens. There is, however, a well-marked tendency for the diploid chromosome number to be restored. This would happen if the first cleavage division of the nucleus were not accompanied by a division of the cytoplasm, and the two daughter nuclei reunited. The imperfections of the spindle mechanisms developed in artificially parthenogenetic eggs seem often to bring this result about. In fact, in some forms such as frogs, similar irregularities in division often occur at later stages of parthenogenetic embryos, and isolated cells or regions of tissue may arise with many different multiples of the basic chromosome number.

SUGGESTED READING

Tyler 1941, 1948, Rothschild and Swann 1949, Runnström 1952*a*.

CHAPTER IV

CLEAVAGE

1. *General features*

In all eggs, fertilisation is followed by a period in which the egg divides into a number of cells. This is known as the period of *cleavage*. It may be said to last until some other process, besides mere subdivision, begins to become important; the process in question is usually a shifting of regions of the egg relative to one another—a series of movements which, as we shall see, will eventually bring about gastrulation. By the time these movements begin the egg has been extensively cut up into smaller cells, and from this time onwards the zygote is commonly referred to as an 'embryo' instead of, loosely, as an 'egg'. As we saw in Chapter I the cleavage cells at the beginning of gastrulation are arranged as a 'blastula', which in its most typical form is a hollow sphere. We shall find, however, that the course taken by the cleavages in many groups is such that the blastula is considerably modified from this pattern.

The main physiological function of cleavage is to redress the balance between the size of the nucleus and the volume of cytoplasm with which it is associated. Egg-cells are always very large, as cells go. During the growth period of the oocyte, the nucleus is also large, being distended to form the germinal vesicle. But after fertilisation, the new zygote nucleus is of about normal size for the species in question, and it finds itself in a cell which is far larger than normal growing cells (although some fully differentiated cells are again very large). During cleavage, the balance is restored. This involves not only a reduction in cell-size by subdivision, but also the formation of new nuclear material to build up the increased number of nuclei. The two most important classes of substance required for these nuclei are the proteins and desoxyribose nucleic acid which together make up the chromosomes. Very little is known of the source of origin of the chromosomal proteins, which cannot easily be isolated from the other proteins of the egg. Technical methods are available for studying the nucleic acids, including the ribose nucleic acid (RNA) which is characteristically present in the cytoplasm as well as the desoxyribose (DNA) compound found in the chromosomes. It was at one time suggested (by Brachet) that in many invertebrates the DNA was formed by conversion of the cytoplasmic RNA (a 'partial synthesis') but this now

seems to be unlikely. It has in fact recently been argued (Zeuthen 1951, Hoff-Jørgensen 1954) that in many forms no net synthesis of DNA takes place during the early part of cleavage, since the cytoplasm of the egg contains stores of this substance which are sufficient to provide for many cleavage nuclei—perhaps a few million in the chick, and a few thousand in the frog, though only about sixteen in the sea-urchin. While this DNA is being incorporated into the nuclei, it is in a state of metabolic activity, since radio-active phosphate is rapidly taken into it (e.g. Villee and Villee 1952); probably also changes are going on in its specificity, converting it into material capable of acting as genetic determinants, but little is known about this.

In the readjustment of the nuclear-cytoplasmic ratio, subdivision of the active protoplasm is of much more importance than cutting up of the inactive yolk, which probably makes no chemical demands on the nucleus. The progress of cleavage is accordingly always profoundly modified by the presence of appreciable quantities of yolk. We find, for instance, that in yolky eggs, the fertilised nucleus is displaced from the centre of the egg towards the less yolky end. Moreover, the cleavages begin earlier and go on faster in the less yolky parts. And very often the cleavage spindles are orientated so as to lie with their axes in the direction of the longest stretch of non-yolky cytoplasm available in the cell—but we shall see that this rule (sometimes known as Balfour's rule) is not the only factor at work in controlling the spindle directions, since these may be definitely orientated even in eggs where there is too little yolk to make Balfour's mechanism effective.

The result of these factors is that in eggs with a fair amount of yolk, the cleavages produce more and smaller cells in the less-yolky animal region than in the more yolky vegetative end. Where the store of yolk is very large, the most heavily laden region may not be divided at all during cleavage; in fact, in extreme cases such as reptile and bird eggs, cleavage is confined to a small superficial area near the animal pole, where alone there is any appreciable quantity of cytoplasm (Fig. 4.1).

We may therefore classify cleavage types as follows:

Total cleavage. Whole egg divides.
(i) *Equal*. In eggs with little yolk.
(ii) *Unequal*. In eggs with rather more yolk.
Partial cleavage. Part of the egg remains undivided; in eggs with rather large stores of yolk.
Superficial cleavage. Cleavage only in a small area of the egg; in extremely yolky eggs.

Total cleavage

Total cleavage is the rule in the small, non-yolky eggs of most marine invertebrates. In the eggs of most species of echinoderms, molluscs, coelenterates, worms, etc., only rather little yolk can be distinguished. The first cleavages in such eggs usually cut them into cells of roughly equal size; such cleavage cells are often known as *blastomeres*. In most cases the equality between them is not very exact, and it usually does not last through many cleavage divisions. Indeed, the most interesting aspect of the cleavage of these eggs is the fact that there are characteristic patterns of large and small cells into which the eggs of a particular group become

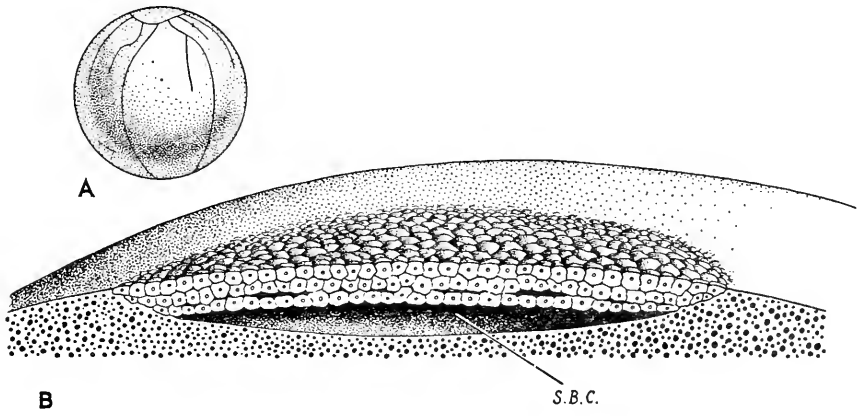

FIGURE 4.1

A. Lateral view of very unequal cleavage in the yolky egg of a sturgeon. *B*. Diagrammatic section of a late cleavage stage in the chick. The blastoderm is beginning to delaminate into two layers, the hypoblast (endoderm) below and the epiblast (ectoderm and mesoderm) above. *S.B.C.* subblastodermic cavity.

divided. In such patterns, there is often no obvious relation between the size of the cell and the yolk content. The cleavage pattern must be determined in some other way (cf. p. 67).

Examples of the main cleavage patterns are described in more detail in the chapters dealing with the different groups. Here we shall merely list them, for reference.

(i) Radially symmetrical. Best seen in echinoderms. In these the first two cleavages are vertical, the third horizontal, but a more complicated pattern begins to appear at the fourth division, in which the cleavage plane is vertical in the animal half and horizontal in the vegetative half, where it cuts off a lower ring of quite small cells, known as *micromeres*.

(ii) Bilaterally symmetrical. In a great many eggs, the first two cleavages are vertical, passing through the animal-vegetative axis, and cut off four cells which are not equal, but arranged with a bilateral symmetry. This is true, for instance, in some coelenterates (e.g. the ctenophore *Beroë*); in ascidians and *Amphioxus;* and in most vertebrates where the accumulation of yolk is not so large as to disturb the picture completely.

(iii) Spirally symmetrical. There is a large group of invertebrates (nemerteans, annelids, molluscs) in which the first two cleavages are not quite vertical, but slightly inclined, so that when looked at from the animal pole, the first four cells are arranged with a slight spiral twist. The third cleavage plane is more or less horizontal, and cuts off a ring of micromeres at the animal end; and these again do not lie immediately above the lower ring of four cells, but are twisted out of place. The subsequent course of cleavage in these eggs has been studied in great detail, particularly by a group of American authors at the beginning of the century (Conklin and E. B. Wilson are perhaps the best known of these). It was shown that the various cells formed after the first five or six cleavages regularly develop into definite parts of the embryo, so that the cleavage pattern is very intimately involved in the developmental processes; we shall see (p. 62) that this is by no means usual in other types of cleavage (Fig. 4.2).

In many animals the simple spiral pattern is modified by a rather remarkable process; just before the first division, the egg pushes out a large pseudopodium-like excrescence, which is called the 'polar lobe' since it forms near the vegetative pole (Fig, 6.4, p. 100). The cleavage runs so that the whole of this lobe becomes incorporated into one of the two daughter-cells. And the process is repeated through several of the later divisions. It appears to be a mechanism for temporarily putting certain material on one side. The cell into which this material eventually comes is the one from which the mesoderm is developed, and thus one of the most important for the future development. Moreover, it has recently been possible to cause an egg with a polar lobe to cleave in such a way that the polar material is divided among the first two cells; it was found that a double embryo was formed (p. 99). This demonstrates the essential role which this material plays in development, and enables us to understand why a special mechanism has been evolved to keep it intact while it is being sorted out into the final mesoderm-forming cell. The result also shows that the spirally cleaving egg is not a true mosaic of parts whose fates are irrevocably fixed, since in the formation of such double embryos a good deal of regulation must have been involved.

(iv) Irregular cleavage. In some coelenterate eggs, the cleavage pattern

is quite irregular; indeed the blastomeres tend to fall apart and are only held loosely together by the jelly in which they are embedded. Perhaps one can also include, under the heading of irregular cleavage, the phenomena found in insects, in which the cleavage consists only in the separation of daughter nuclei within the undivided mass of the yolky egg (see p. 119).

Unequal, partial and superficial cleavage

The most important examples of unequal cleavages are found in vertebrates, which present a complete series of types, from those in which

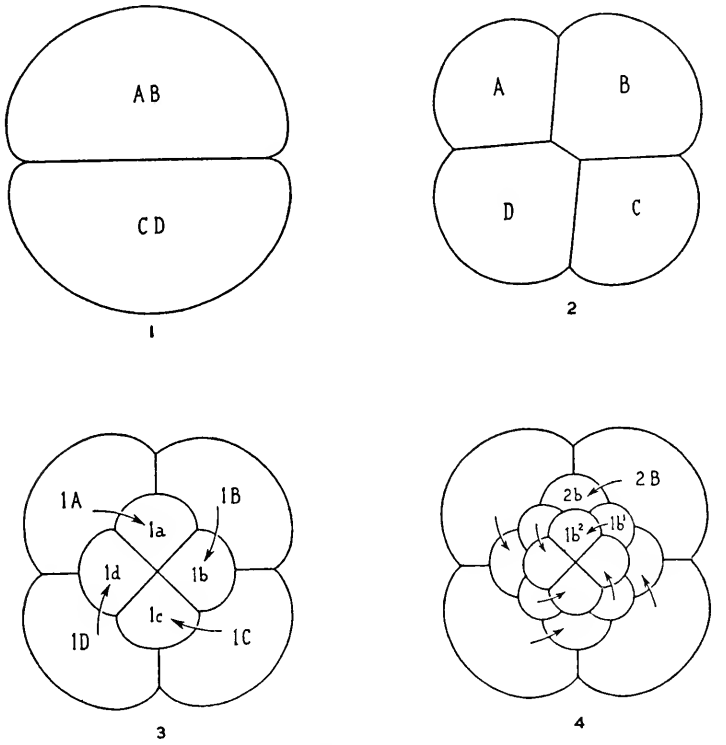

FIGURE 4.2

Spiral cleavage (dextral) seen from the animal pole. At the 4-cell stage, the right-or left-handedness of the cleavage can already be recognised by the direction of the cross-furrow at the animal pole. At the third cleavage, the macromeres *A*, *B*, *C*, *D* give off micromeres 1*a*, 1*b*, 1*c*, 1*d*, the cleavage spindles being tilted in the direction indicated by the arrows. At the next cleavage, the second quartet of micromeres, 2*a*, 2*b*, etc., are formed, with the spindles tilted in the other direction; and 1*a* divides into 1*a*1 and 1*a*2, 1*b* into 1*b*1 and 1*b*2, etc. This system of cleavage continues until four quartets of micromeres have been formed; but the divisions of the micromeres and macromeres are not always synchronous, cleavage of 1*a* into 1*a*1, 1*a*2 being sometimes delayed till after the formation of the second quartet, and so on.

there is only a minor difference between the animal and vegetative cells, to those in which cleavage is confined to a relatively tiny area at the animal pole. The frog or newt are good examples of slightly unequal cleavages, some of the cartilaginous fishes are the best intermediate types, while the reptiles, teleosts and birds are the classical 'superficial' types.

In the more primitive, nearly equal, members of this series (such as Amphibia) the pattern seems to be one of bilateral symmetry, the first two cleavages being vertical, and forming four cells of which one pair is slightly larger than the other. The third cleavage is horizontal, but lies above the equator, so that the less yolky animal cells are smaller than the vegetative ones. This difference is greatly exaggerated in the more yolky eggs, such as those of sturgeons. But in the most yolky eggs there is a sharply different type of cleavage. The small animal cells do not gradually shade off into larger and larger blastomeres; instead, the cleavage occurs only in the animal cytoplasmic region, in which all the cleavage cells are of similar size, while the main mass of yolk remains completely undivided. There is thus a rather sharp boundary between the cellular and non-cellular parts of the egg. (There may be a few nuclei scattered in the yolk just near the border of the cellular region, these being derived from supernumerary sperm which enter the egg outside the sphere of influence of the primary fertilising sperm.) This is the condition in reptiles and birds.

In a very rough way, the series from non-yolky, totally cleaving eggs up to very yolky, superficially cleaving types parallels the evolutionary series of the vertebrates. But the parallel breaks down entirely when we come to the mammals. Since it is nurtured in the uterus, the mammal egg has no need of large stores of yolk, and in fact is not provided with them. And in the absence of yolk, the cleavage is total and more or less equal, though with no very well-defined symmetry pattern.

2. *The pattern of cleavage and the pattern of the embryo*

Since the cleavages frequently follow a definite and orderly pattern, it is perhaps natural to expect that this will be directly related to the pattern of the embryo which eventually develops. Many of the earliest studies on the physiology of development aimed at discovering whether this is so or not. It turns out that there is no simple, single answer which applies to all animals; in fact this is a question to which we shall have to return several times in the later discussions of the development of different groups. Even the very earliest experiments, at the end of the last century, showed that the subject was complicated. Driesch, in 1891, separated the first two blastomeres of an echinoderm egg, and found that each gave rise to a complete embryo, not to only half an embryo as would be expected

if there is a direct relation between cleavage pattern and embryo. He concluded that any part of the egg, in the early cleavage stages, is capable of forming a whole animal; and he spoke of such eggs as 'regulation' eggs, all of whose parts are 'equi-potential' and capable of regulating so as to replace any part that might be removed. The critical reader will notice that Driesch drew conclusions about the behaviour of all parts from experiments in which he had actually only succeeded in isolating certain parts; thus he always cut the egg parallel to the first cleavage planes, which are vertical, and he was not justified in assuming that if he had cut it along a horizontal plane, those halves would also regulate completely. As we shall see later, in fact they do not do so; and it is probable that no egg is actually a completely regulation egg in Driesch's sense.

Very soon after Driesch's discovery, similar experiments on other eggs turned out in exactly the opposite way. Roux, for example, found that when one of the first blastomeres of a frog's egg is killed, the other develops into half an embryo; and the same thing occurred in ascidians and many of the spirally cleaving eggs. Embryologists began to speak of 'mosaic' eggs, contrasting these with the regulation type, and supposing that they contained a mosaic of different cytoplasmic regions each of which irrevocably developed into some specific part of the embryo. But in this concept again they were going beyond their actual facts. The experimental results showed that when certain eggs were injured, the remaining parts *did not* regulate so as to compensate for the loss; but this does not necessarily imply that all regulation is impossible in such eggs. We shall see later that this is not only a logical *non sequitur*, but is controverted by the facts which have become known more recently. In fact, there seems to be no more a completely mosaic egg than a completely regulation one. All eggs, we shall find, partake of both characters; all have some definiteness of localisation of parts with specific properties; and all have some capacity for adjusting themselves to injuries. It is true that in some eggs the localisation is the more striking phenomenon, in others the regulation; and many eggs can still be looked on as tending to one or other extreme; but the differences are not absolute, and the two pure types do not exist.

3. *Differentiation without cleavage*

In the early years of the century F. R. Lillie discovered that parthenogenetically activated eggs of the polycheate *Chaetopterus* may sometimes achieve a considerable degree of differentiation although remaining in an undivided state. In these eggs the cleavages are, however, not totally suppressed. Pasteels (1934) and Brachet (1937), who have re-studied the material more recently, point out that the activated egg becomes lobulated

in a manner which closely simulates the normal cleavage pattern, and that there are cycles of activity of the nucleus, involving the appearance of asters, usually monocentric but occasionally leading to true mitoses. Although the lobulations eventually disappear, so that the egg regains its spherical shape, their occurrence is important evidence that autonomous cytoplasmic (probably cortical) changes play a part in the normal cleavage process (see also Lehmann 1948*a*).

The 'differentiation' performed by these eggs is also by no means complete. The most they accomplish is the separation into different regions of various types of cytoplasm, together with a relative movement of the outermost clear cytoplasm over the vegetative material which somewhat recalls the normal process of gastrulation, and finally a differentiation of cilia; but there is no true organogenesis, such as the formation of a gut or apical tuft. Nevertheless, the evidence that even this segregation of ooplasmic components can proceed as far as it does when the egg is not divided up into cells, indicates the importance of processes which go on within the body as a whole. It becomes clear that it is dangerous to attribute too much importance to the cell as the basic unit on which everything depends, and that theories such as that of Weiss (p. 413) which attempt to explain development in terms of the properties of cell membranes can be at best a part of the truth.

4. *Cleavage without nuclei*

It has been mentioned in the last chapter that parthenogenetically activated eggs or egg fragments may undergo fairly regular cleavages even in the absence of nuclei. Cases of this have been described by Harvey (1936, 1940) in echinoderms, by Gross (1936) in Artemia, and Fankhauser (1934) and Briggs, Green and King (1951) in Amphibia. Some authors question whether the phenomenon is truly comparable to cleavage, and suggest that it is more like the disorganised 'bubbling' that some cells undergo at the time of division, but in the best cases described in the Amphibia something very like a normal blastula is produced, and it seems unduly sceptical to deny the process the name of cleavage (Fig. 4.3).

5. *The mechanism of cleavage*

Cleaving eggs, particularly those of marine invertebrates, have frequently been used to study the general problems of cell division. This is an enormous subject, involving the behaviour of the chromosomes, of the achromatic apparatus (spindle, centrosomes, asters, etc.) and of the body of the cell which becomes divided in two. A full discussion of all aspects of it would lead us too far into the fields of cytology and cell

physiology and we shall confine ourselves here to those aspects which are particularly important in relation to the problems of embryonic development. We shall therefore pay little attention to the subject of chromosome movements or the details of the behaviour of the achromatic apparatus (for which consult textbooks on cytology, such as Darlington 1939, White 1950, 1954, Schrader 1944, Hughes 1952). From the point of view of the embryologist, the important subjects to discuss are the determination of the pattern of cleavage and the mechanism by which the body of the cell becomes divided into two parts.

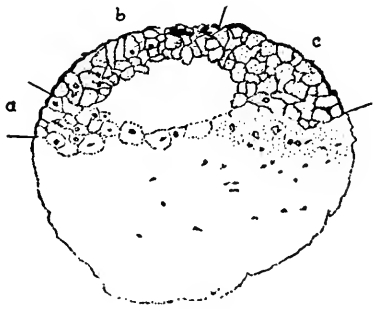

FIGURE 4.3

Section through the best cleaved portion of a blastula, which was developed from an enucleated egg of the frog *Rana pipiens*, inseminated by a sperm of *R. catesbiana* whose nucleus had been inactivated by U.V. irradiation. The cells are outlined by pigment granules and the dark spots resembling nuclei are also accumulations of pigment. There is some chromatin in most cells in region *a*, in a few cells in region *b*, but none in the cells of region *c*. (From Briggs, Green and King 1951.)

The series of events by which a cell is cleaved in two is normally initiated by the nuclear division, during which the chromosomes become separated into two daughter groups. That there is a causal relation between the two processes is shown by the general concordance in their timing: nuclear division is usually followed immediately by cell division. The primacy of the former is shown by the fact that when, in abnormal cells or under experimental conditions, the usual sequence is disturbed, it is the cleavage of the cell rather than the division of the nucleus which is most easily changed from its normal course. Thus it is not uncommon for nuclear divisions to occur without any following cleavage of the cell body, but it is very rare for the cell to cleave in a way which is not dependent on the events proceeding in the nucleus. We do not, for instance, find a cell cleaving before the nucleus has entered into its division process, or a

cell membrane cutting through a spindle before the separation of the chromosomes has occurred. The dependence of cell division on nuclear phenomena is, however, not absolute, since it can occur in parts of cells which contain no nucleus (p. 64). But in these anucleate cells, centrosomes, if missing, arise *de novo*, and it seems that they control the occurrence of the cleavage; thus even in this case the cytoplasmic division is not wholly independent of the behaviour of the achromatic apparatus.

The nature of the connection between the nuclear division and the cleavage of the cell body is not well understood. Swann (1951, 1952) has showed by studies with the polarising microscope that the orderly arrangement of the material which forms the asters at each pole of the spindle decreases as the chromosomes come into their neighbourhood at anaphase. He suggests that this is brought about by a substance released from the chromosomes, and that this substance diffuses away from the two daughter-nuclei until it reaches the cell cortex, where it initiates the processes leading to cell cleavage. It would seem that some diffusing agent of this kind must almost certainly be involved, but it is not clear that it arises from the chromosomes.

Darlington (1937) has drawn attention to the type of cell cleavage which occurs in cases where the movement of some of the chromosomes on the spindle has been abnormal. He claims that if one or two chromosomes have lagged behind the others and become included in a small separate nucleus of their own, a plane of cell division often forms around this nucleus, but only in those cases in which the chromosomes are provided with centromeres: the cleavage planes pay no attention to acentric fragments. Again, it is well known that if two homologous chromosomes become joined together (e.g. by chiasmata inside an inversion) and are unable to separate properly at anaphase, they form a 'chromosome bridge' connecting the two telophase nuclei, which move away from one another as far as the connection will allow. The cleavage plane then forms between the two nuclei but is unaffected by the chromosome bridge and cuts through it as though the connecting chromosome material were not present. From this type of evidence Darlington concludes that it is the centrosomes and centromeres which affect cell cleavage rather than the chromosomes themselves.

The first factor, then, which plays a part in the determination of the cleavage pattern of an egg is the orientation and position of the cleavage spindles which initiate the changes in the cell body. In spirally cleaving eggs, for instance, from the 4-cell stage onwards the cleavage spindles are obliquely inclined, first to one side and then to the other of the vertical, and this gives rise to the characteristic pattern of the group of cells. The

nature of the factors which in their turn determine the orientation of the spindles is unknown, but there must be some sort of continuous change proceeding within the cytoplasm which controls their development. The occurrence of such changes is well shown by some experiments of Hörstadius (1939) on the echinoderm egg. He used eggs of the sea-urchin *Paracentrotus lividus*, which possesses a sub-equatorial band of pigment, which makes it possible to recognise the orientation of the egg even when the cleavage is abnormal. By treatment with hypotonic sea water or by shaking, one can cause a delay in the appearance of the cleavage furrows, but the results show that the factors controlling the orientation of the spindles go through their usual changes at the normal rate. Thus the first cleavage may be delayed until the spindle mechanism is ready for the second cleavage, and the next until it is intermediate between the normal second and third (Fig. 4.4, second row).

This same experiment also shows that the orientation of the spindle is not the only factor concerned in the cleavage pattern. In the *Paracentrotus* egg the vegetative region after a certain time has a tendency to form very small cells (micromeres), and will do so whatever the orientation of the spindle which initiates the division (Fig. 4.4, row 3). Again, spontaneous constrictions, mimicking the early stages of cleavage, are seen in the

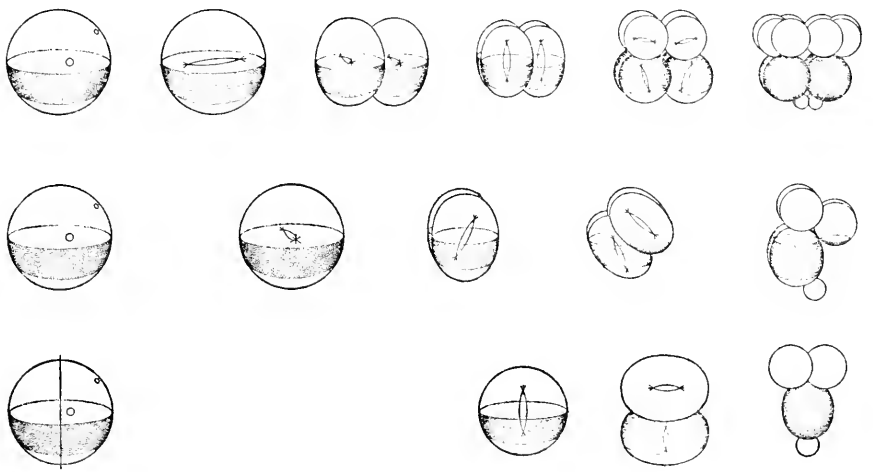

FIGURE 4.4

Delay of cleavage relative to the orientation of the spindles. Upper row, the normal cleavage of the echinoderm *Paracentrotus*. Middle row, cleavage somewhat delayed. Lower row, cleavage further delayed, the first cleavage not occurring till the spindle is orientated vertically. (From Hörstadius 1937.)

parthenogenetic eggs of *Chaetopterus* which 'differentiate without cleavage' (p. 63), and in isolated and non-nucleated polar lobes of molluscs (Morgan 1933). The operative agent in these instances is almost certainly located in the egg cortex.

There are many other instances in which it can be shown that the local properties of the cortex influence the course of the cleavage planes. This factor is of importance in nearly all eggs in the formation of the small polar bodies. Morgan (1937) has tried to discover why the maturation spindles in the egg normally give rise to such extremely unequal divisions of the cell body. He showed that in the marine snail *Ilyanassa* the second polar-body spindle could be shifted into the middle of the egg by centrifuging, and that in this position, when it is no longer near the polar cortex, it is capable of causing the egg to divide into more or less equal parts. It only does so if the egg is still somewhat elongated after the centrifugation; if the egg becomes completely rounded up again, a centrally-placed maturation spindle fails to cause it to divide. In the parthenogenetically activated *Urechis* eggs studied by Tyler (p. 54), the displaced polar-body spindles seem to be more effective and able to cause an equal division even of a spherical egg, provided they have been shifted away from the polar cortex.

Another clear example of the influence of the cortex is provided by the experiments of Lehmann (1946) on the freshwater oligochaete *Tubifex*. In this form, considerable protuberances are pushed out from the body of the cell, both at the first and second polar-body divisions and at the first cleavage division (Fig. 4.5). In the polar-body divisions, which occur with the spindle near the animal pole, the protuberances are arranged in a more or less symmetrical manner around the animal-vegetative axis. The first cleavage division is unequal and gives rise to a small *AB* cell and a large *CD* cell. The protuberances at this division form mainly at the equator of the cell, in particular in the region of the *AB* blastomere. They are thus arranged bilaterally symmetrically as seen from the animal pole. By moderate centrifugation the spindle of the second polar-body division can be moved from its normal position without the structure of the cortex being materially affected. If the egg is arranged so that the centrifugal force is parallel to the animal-vegetative axis, the internal contents of the cell are stratified and the polar-body spindle moved from the animal pole into the interior. It is found that the pattern of the protuberances which form at the next cell division is hardly altered, even if the spindle has been moved right down to the vegetative pole of the egg. If, however, the egg is orientated so that the centrifugal force is at right-angles to the axis, the spindle is shifted towards the egg equator on one side. With this orienta-

tion the egg becomes much more elongated, with a given degree of centrifugation, than it does when the force acts along the egg axis; this presumably indicates that the egg cortex is more easily deformed in the equatorial than in the animal-vegetative plane. When second polar-body formation begins, protuberances appear in the equatorial region of the egg in the neighbourhood of the spindle. They thus form a bilaterally symmetrical pattern very similar to that characteristic of the normal first cleavage, in which again it is an equatorial region of cortex which is closest to the initiating spindle. It is clear, then, that the protuberances are

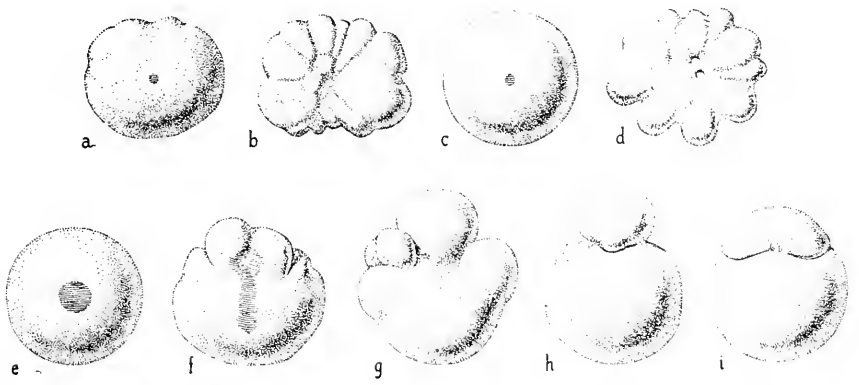

FIGURE 4.5

Maturation and first division of the egg of *Tubifex*, seen from the animal pole; *a*, soon after laying; *b*, formation of protuberances during the extrusion of the first polar body; *c*, between first and second polar body divisions; *d*, extrusion of the second polar body; *e*, before first cleavage of fertilised egg (the animal pole plasm shaded); *f*, early stage of cleavage, pole plasm elongated; *g*, *h*, stages of cleavage showing protuberances; *i*, two cell stage (the *AB* blastomere above, *CD* below). (After Woker 1944.)

produced by an interaction between the cleavage spindle and the cortex in its neighbourhood, which has a structure which differs in the different parts of the egg. Lehmann claims that this structure is to some extent visible in the living egg, which shows a pattern of nine to fifteen subcortical meridianal thickened strands of the heavy fibrillar type of cytoplasm which he names 'plastin'. This material is, however, at least to some extent, shifted by the centrifugation, and the structure which persists in the cortex of the centrifugal eggs is perhaps not due to the plastin, but to some associated structure in the cortex itself.

There is normally also an accumulation of plastin around the nucleus. With mild centrifugation in a polar direction at a stage shortly after fertilisation, all the plastin is driven to the centrifugal end while the nucleus still remains near the centripetal end, surrounded only by yolky cytoplasm. In such eggs the nucleus shows no sign of entering into division, which presumably indicates that the nuclear processes are normally initiated by a reaction which involves the surrounding plastin material. Further, no deformations of the cell cortex occur, which again demonstrates that they are initiated by the nuclear division process. These interactions between cortex and nucleus in *Tubifex* are somewhat reminiscent of those between the nucleus and the cortical granules shortly after fertilisation in the echinoderms, as described by Allen (p. 51).

It appears, then, that the pattern of cleavage is determined by interactions between the spindle and the cortex. We have now to consider the nature of the forces which bring about the deformation of the cell and its division into two. There are a number of theories in the field. As the first group we may take those which suppose that the nuclei and spindle apparatus continue to play in important part throughout the whole course of the division. For instance, Gray (Review: 1931), from studies on the sea-urchin egg, suggested that the asters continue to grow until all the available cytoplasm is incorporated into two spheres of radially arranged material at the poles of the spindle, and that the cortex passively accommodates itself to these two masses, which form the two first blastomeres. Dan (Review: 1948), working on the same form, suggests that the astral rays are actually attached to the cortex, and he shows by means of a model how, if this were so, a contraction of the rays would cause the bending in of the cortex and at least the beginning of the process of division. However, these mechanisms certainly do not operate in all types of eggs, if indeed in any. For instance, cleavage in the amphibian egg can continue quite satisfactorily when a large amount of the internal contents has been removed so that the cortex is quite flaccid and the asters unable to produce any internal turgor. Moreover, after a cleavage furrow has begun to form it can extend over a region of cortex which has been isolated from the spindle by the insertion of a strip of cellophane, which must certainly prevent the attachment of any astral rays (Waddington 1952*d*; Mitchison 1953 has similar evidence in echinoderms). (Fig. 4.6.) Finally Swann and Mitchison (1953) find that if sea-urchin eggs are treated with colchicine at anaphase, when the chromosomes have separated but the division of the cell body has not yet begun, the asters disappear but the cleavage occurs normally. Thus it seems fairly certain that although the division spindle initiates the process of cleavage it does not play any straight-

forward mechanical part in carrying the process through to completion. It seems that the main active agent must be the cortex itself.

Probably the most widely accepted theory is one which ascribes the cleaving of the cell to the contraction of a ring of cortex around the position of the future furrow. This theory, which has recently been defended by W. H. Lewis (1951) and Marsland (1951, Marsland and Landau 1954) is the one which suggests itself most naturally when one looks at dividing

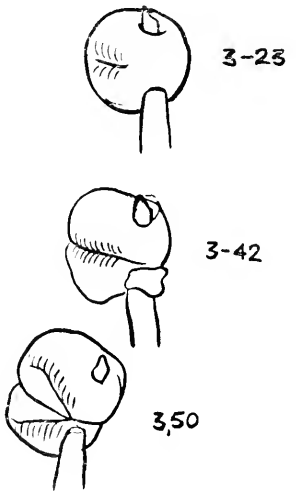

FIGURE 4.6

The independence of the cleavage furrow, once it has started, of contact with the spindle. In a newt's egg which was just starting to cleave at 3.23 p.m., a strip of cellophane was inserted under a region through which the furrow should extend. Within the next half-hour it did actually extend through the area. (From Waddington 1952*d*.)

cells and tries to think how their behaviour might be explained in terms of cortical activity. There is, however, rather little direct evidence for it. And, as Mitchison (1952) points out, there are some difficulties in it when one examines the matter more closely. In the first place, if the cell is to be constricted completely into two parts, the ring of contracting material would have to contract away to nothing. This, Mitchison argues, would seem to be an unlikely event, if one thinks of it in terms of a contraction like that of muscle: it is perhaps not so unplausible if one pictures the contracting ring as similar to the ectoplasm at the posterior end of an advancing amoeba, which liquefies after it has finished contracting. Again,

when a cell divides its surface area must increase; in the case of a spherical egg becoming converted into two spheres, the increase is about 26 per cent. A contraction of the ring of the furrow would therefore have to be compensated by a large increase in area elsewhere. The main evidence on which Marsland relies, for support of the 'contracting ring' hypothesis, is the demonstration that if gelation of the cortex is prevented (by high hydrostatic pressure or low temperature) the development of the cleavage furrow is inhibited. This might be due, as he suggests, to an abolition of the contraction; but it might equally be a consequence of the failure of the ungelated cortex to transmit the stress from the expanding regions to the furrow in the way required in the mechanism postulated by Mitchison and Swann. Thus the evidence is not fully conclusive.

Mitchison and Swann (1955, see Mitchison 1952) have recently turned the conventional theory upside down and suggested that the prime mover in cell cleavage is not a contraction of the furrow region but an expansion of the other parts of the cortex. They suppose that the cleavage process is initiated by substances released by the two separating groups of chromosomes (there seems no reason why one should not attribute the activity to the centromeres, rather than to the chromosomes themselves, in accordance with the points made on p. 66). This substance would diffuse out into a more or less dumbbell-shaped region which, in a spherical egg, would reach the cortex first at the equator opposite the poles of the spindle (Fig. 4.7). The substance is supposed to cause an expansion of the cortex, and this would begin in the same region. Meanwhile the ring of cortex which lies in the plane of the equator of the spindle (and therefore in-

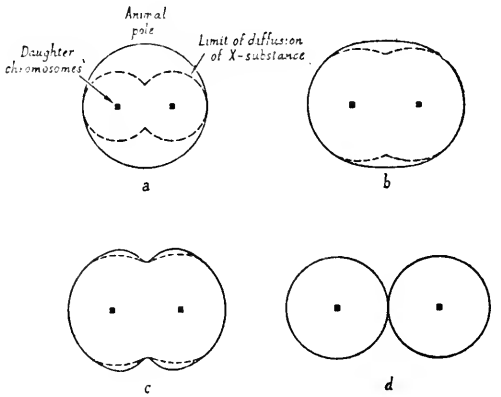

FIGURE 4.7
Diagram of cleavage in the sea-urchin. (After Mitchison 1952.)

cludes the poles of the egg) will become flattened for two different reasons. Firstly, Mitchison claims that there seems always to be some tension in the egg cortex, so that when it expands in one place, the other regions will tend to contract; and secondly, the expansion of the cortex on the *egg* equator is supposed actually to push the rest of it towards the plane of the *spindle* equator[1]. These two processes will, in fact, cause the future furrow not merely to flatten but to bend inwards. This will bring it in contact with the expanding dumbbell-shaped area containing the diffusing active substance, and when this happens the cortex in the furrow will be acted on by the substance and caused to expand; and this expansion completes the formation of the cleavage plane.

This theory was worked out in the first place from studies on echinoderm eggs. In that form there is a fair amount of support for various parts of it. Thus Mitchison and Swann have presented evidence that the cortical changes leading to cleavage begin in the equatorial regions of the egg, opposite the poles of the spindle, in the form of a decrease of the birefringence and light-scattering properties of the cortical material. Although the structure of the cortex is not at all well understood, it is reasonable to suppose, as Mitchison suggests, that it is made up largely of protein chains folded at right-angles to the surface, and that the changes in birefringence are connected with an expansion in area. Again, Dan and Ono (1954, see Dan 1948, 1954) have followed the movements of small particles of kaolin attached to the cell surface. They found that there was an expansion starting opposite the poles of the spindle and spreading towards the future furrow. The furrow region itself at first contracts somewhat, and then expands greatly as the new plane of division cuts down into the depth of the cell.

Finally, Mitchison and Swann (1955) have devised an apparatus for measuring the deformability of the cell surface. The tip of a small pipette is brought against it and a negative pressure or suction applied; one can then measure the height of the small protuberance which the suction raises on the surface. With this apparatus they showed that shortly before division the cortex becomes much less easy to deform. They suggest that this is not due to an increase in a tension (such as a surface tension) in the cortex, but rather to that layer becoming stiffer or less plastic. They argue that this makes it easier to suppose that the expanding regions opposite the poles of the spindle can successfully push the rest of the cortex into the furrow region.

[1] The student is warned to beware of the verbal pitfalls that can occur from the fact that the long axis of the first cleavage spindle is perpendicular to the animal-vegetative axis of the egg.

Raven (1948) has estimated the 'tension at the surface' of the eggs of *Limnea* from fertilisation till first cleavage. His method was to centrifuge the egg for 5 minutes at a moderate speed, which gave a force of 1860g. Observation of the distinctness with which the internal contents become stratified gives an indication of the viscosity of the cytoplasm, while the extent of the elongation of the egg allows one to draw some tentative conclusions about the deformability of the surface. The elongation will, however, also be affected by the degree to which the internal constituents separate into definite layers, so that the viscosity changes somewhat obscure the picture of the alterations in the properties of the surface. Raven finds that the viscosity is low immediately before each of the first three cleavages, but rises as the furrow makes its appearance, and reaches a maximum about 10–15 minutes later. The data on the surface tension are not so clear cut, and only the first cleavage has been investigated. Raven finds that it is low immediately before the appearance of the furrow. His diagrams show, although he does not refer to the fact in discussing them, that it rises steeply during the cleavage. It is not quite clear whether this rise occurs at a slightly later period than the similar increase in stiffness of the cortex of the echinoderm egg, but, apart from possible minor changes in timing, the phenomena seem rather alike in the two groups.

These results are good evidence that some, at least, of the processes envisaged in Swann and Mitchison's theory actually occur. Moreover there is plenty of other evidence that expansions of the cell membrane are often associated with cleavage. Some of the most extreme examples of this are seen in those spirally cleaving eggs in which a polar lobe is formed (Fig. 6.2). But the mere occurrence of an expansion does not suffice to show that it is the prime mover in bringing about a cleavage of the cell, and there is some reason to doubt whether it is more than one out of a number of factors which may play a part. For instance, we have seen that in *Tubifex* there are considerable cortical expansions which cause the egg to throw out protuberances at the times of polar-body division and at the first cleavage division. The second cleavage division occurs first in *CD* blastomere and only somewhat later in the *AB* one, and is not accompanied by such a pronounced cortical expansion as the earlier ones, though the *CD* cell does elongate considerably and increase its surface area to a fair extent just before the cleavage occurs. Huber (1947) has studied the action of two anti-mitotic substances, naphthoquinone and phenanthrenequinone. He finds that the former has a particularly strong effect on the expansions, tending to suppress the formation of protuberances in the first division and the stretching of the *CD* cell in the

second. The second substance, on the other hand, leaves these processes relatively unaffected or even exaggerates them, but the cells tend to fail actually to divide into two, so that one finds, for instance, an elongated but undivided *CD* cell (Fig. 4.8). Huber therefore concludes that cell division involves two different cortical movements, not only the expansion seen in the formation of protuberances, but also a contraction in the furrow region.

A consideration of more yolky types of egg would also suggest that a mere expansion of the already existing cell cortex cannot be the only factor causing the cleavage. In the extremely yolky eggs of a sturgeon,

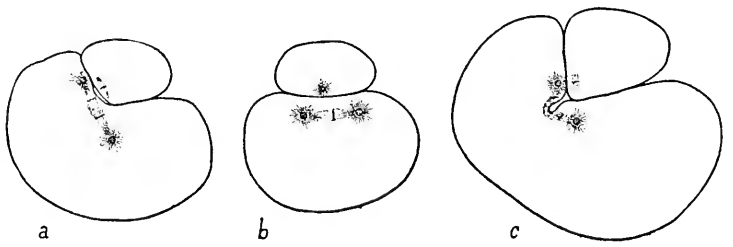

FIGURE 4.8

The effect of quinones on cleavage: *a*, section through a 2-cell stage of the egg of *Tubifex;* the nuclei are already in telophase of the second division and the *CD* cell (below) is markedly elongated preparatory to dividing in two; *b*, a similar stage from an egg treated with 1,4-Naphthoquinone, note the slight elongation of the *CD* cell; *c*, after treatment with 9,10-Phenanthrenequinone the elongation of the *CD* cell is exaggerated. (After Huber 1947.)

for instance, the cleavage furrows eventually extend a long way from the position of the spindle, and it is difficult to see how the influence of a cortical expansion starting in the neighbourhood of the spindle poles could reach so far (cf. Fig. 4.1). Moreover in such forms the second cleavage furrow starts to form in the animal region while the first furrow is still working its way down towards the vegetative part of the egg. It seems unlikely that any system of expansions working through the cortex as a whole could control the furrows in such cases. It would be much easier to attribute the formation of the cleavage planes to factors located in the immediate neighbourhood of the furrows themselves.

Studies on the moderately yolky eggs of the Amphibia suggest, indeed, that in them also cortical expansion is not such an important factor in cleavage as Mitchison and Swann suppose it to be in the smaller eggs of the echinoderms. Selman and Waddington (1955) have shown that there

is little movement of cortical pigment granules in the neighbourhood of the poles of the spindle and there is no noticeable cortical expansion there. However there is a considerable flow of the granules along the furrow as it first appears near the animal pole of the egg. This movement along the furrow would seem to indicate a contraction acting in this direction and at this place. Moreover if the eggs are viewed from the side it can be seen that just before division the whole cell heaps itself up so that its vertical height increases (Fig. 4.9). This movement is accompanied by an increase in the resistance of the cortex to deformation as measured by

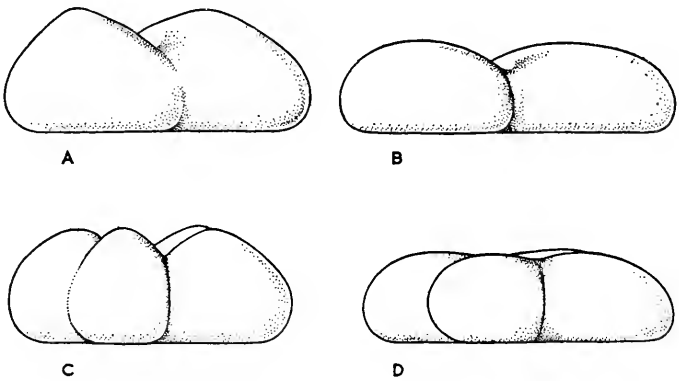

FIGURE 4.9

Side views of cleavage in a newt's egg removed from its vitelline membrane to show the 'rounding up' at the beginning of division. *A*, part-way through the first division; *B*, between first and second division; *C*, beginning of second division; *D*, second division completed. (After Selman and Waddington 1955.)

the suction apparatus of Swann and Mitchison. This must involve a force acting in the cortex, which might be either analogous to a surface tension, or, more probably, due to an increase in the elastic constants of the material. In any case, the rising up of the egg suggests a decrease in surface area rather than an increase. It becomes rather unplausible, then, to attribute much importance to cortical expansion in this case; we seem rather to have to deal with a localised contraction along the length of the developing furrow.

There is, however, almost certainly another factor involved. When an amphibian egg cleaves, the surface between the two blastomeres is almost unpigmented, and thus differs sharply from the cortex of the

uncleaved egg. It seems that it must have arisen *de novo*. Schechtman (1937) suggested that it is formed by the growth of the cortex into the depth of the furrow. Mitchison and Swann argue that such growth takes place, not merely from the very edge of the original cortex, but by intussusception of new material throughout the whole area of the infolding region; if this were so, the process becomes in effect a type of cortical expansion.

A similar increase in surface area occurs in the furrow region of the echinoderm, but in this form it seems to take place after the cleavage plane has cut through the egg (Fig. 4.10). According to Dan (1954)

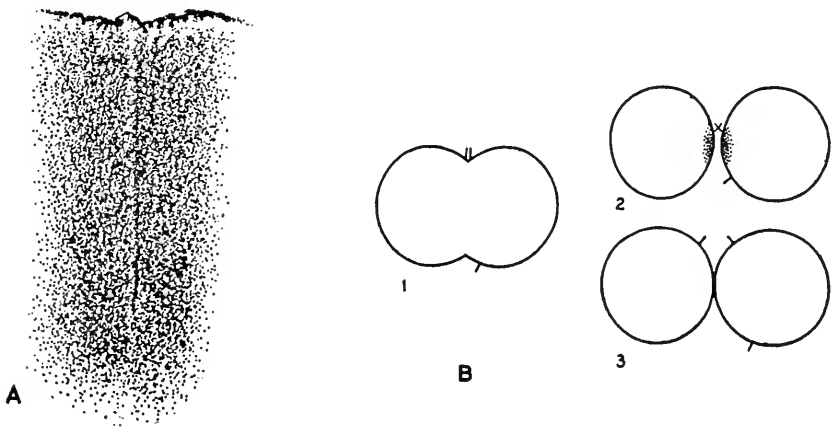

FIGURE 4.10

A. Section through part of a newt's egg at an early stage in the first cleavage. The future surface which will separate the two blastomeres is already indicated in the internal cytoplasm although there is not yet any furrowing of the external surface in this region. (After Selman and Waddington 1955.) *B*. Three stages in the cleavage of an echinoderm egg. Small kaolin particles (indicated by short lines) have been placed on the surface. Note that they at first move into the furrow (*2*), but that later new cortex is produced in the depth of the furrow (dotted), so that the particles move out again. (From Dan 1948.)

it also seems to be due to intussusceptive growth, accompanied by stretching. From this evidence, Mitchison and Swann conclude that the cortical expansion involved in their theory always includes an element of intussusceptive growth. But even if this is so, the amount of growth as compared with mere stretching must be so vastly greater in the furrow region than elsewhere as to amount to a qualitatively different type of

phenomenon. Moreover, Selman and Waddington find that sections of an amphibian egg in the process of division show that an indication of the future cleavage plane is developed in the cytoplasm over a much wider area than corresponds to the externally visible furrow. It seems very unlikely that the material making up this precursor of the division plane is directly derived from the original cortex. It looks rather as if it differentiates *in situ* (although it must be the cortex which initiates the process of differentiation). It gradually increases in extent, and also in thickness, and eventually splits into two separate films which form the outer membranes of the two blastomeres in the region where they are in contact. If the vitelline membrane is removed, so that there is nothing to hold the two cells together, they tend to fall apart as they settle down on the bottom of the dish, and the white newly formed cell membrane is then extensively exposed in the depths of the furrow as one looks at the egg from the animal pole; it may be visible within the furrow even in eggs cleaving inside the membrane.

The evidence available at the present time would seem, therefore, to suggest that several factors are operative in the process of cell cleavage, their relative importance differing in various groups of animals. These factors are: firstly, localised expansions of the cortex; secondly, an increase in stiffness of the cortex; thirdly, in the Amphibia at least, an increase in the tangential force acting in the cortex; fourthly, a contraction localised in the neighbourhood of the furrow and directed along its length; fifthly, a formation of new cell membrane from the sub-cortical cytoplasm. It is possible that Swann and Mitchison are correct in ascribing the major importance to the first two of these in the echinoderm egg but it seems likely that in other eggs, particularly those with large quantities of yolk, the other three factors have to be taken into account.

SUGGESTED READING

Fankhauser 1948, Mitchison 1952, Swann 1952, Lehmann 1946.

CHAPTER V

ECHINODERMS

1. *Normal development*

The early stages in the development of echinoderm eggs are very simple, and provide classical examples of the two fundamental forms, the blastula and gastrula. Moreover, the physiology of these stages has been rather fully investigated. There is therefore much of interest in echinoderm development even for a general account of embryological principles, in spite of the fact that the later stages are highly complex. The simple, early stages of development lead to the formation, not of the adult, but of a larva, which is usually the so-called 'pluteus'. The insertion of a larval stage into the life-history is, of course, very common among invertebrates. Such larvae fulfil many functions; they may, for instance, facilitate the dispersal of the species, as in many groups of parasites; or they may seem to have been evolved as the quickest way in which the egg can be converted into an animal capable of feeding itself. The latter would appear to be the *raison d'être* of the Pluteus; it is a little animal which can swim by means of cilia, and feed itself from the minute life among the plankton. It is only after a considerable independent existence that it becomes converted, by a complicated metamorphosis, into the adult. In this discussion, we shall not attempt to deal with anything more than the first steps of development, by which the larva is produced (Reviews: Lehmann 1945, Hörstadius 1939, 1949).

The cleavage of the echinoderm egg is total, and radially symmetrical. The first two cleavages are vertical, through the animal pole. The third is horizontal, running slightly above the equator, so that the upper four cells are rather smaller than the lower four. From this point onwards, the cleavages in the animal and vegetative halves take different courses. At the fourth cleavage, the animal cells divide into a flat ring of eight, while the vegetative ones cleave very unequally into two rings, four large 'macromeres' above and four tiny 'micromeres' below; the cleavage planes are thus nearly at right angles in the animal and vegetative halves. The same is true at the next cleavage, but here it is in the animal half that the division is horizontal, while in the vegetative half it is more or less vertical. At the sixth cleavage, all the division planes are horizontal, and thus we come to a stage with four rings of animal cells, two rings of macromeres, and two of micromeres, there being eight cells in each ring.

For our present discussion, it is not worth while tracing the cleavage in detail beyond this point (Fig. 5.1).

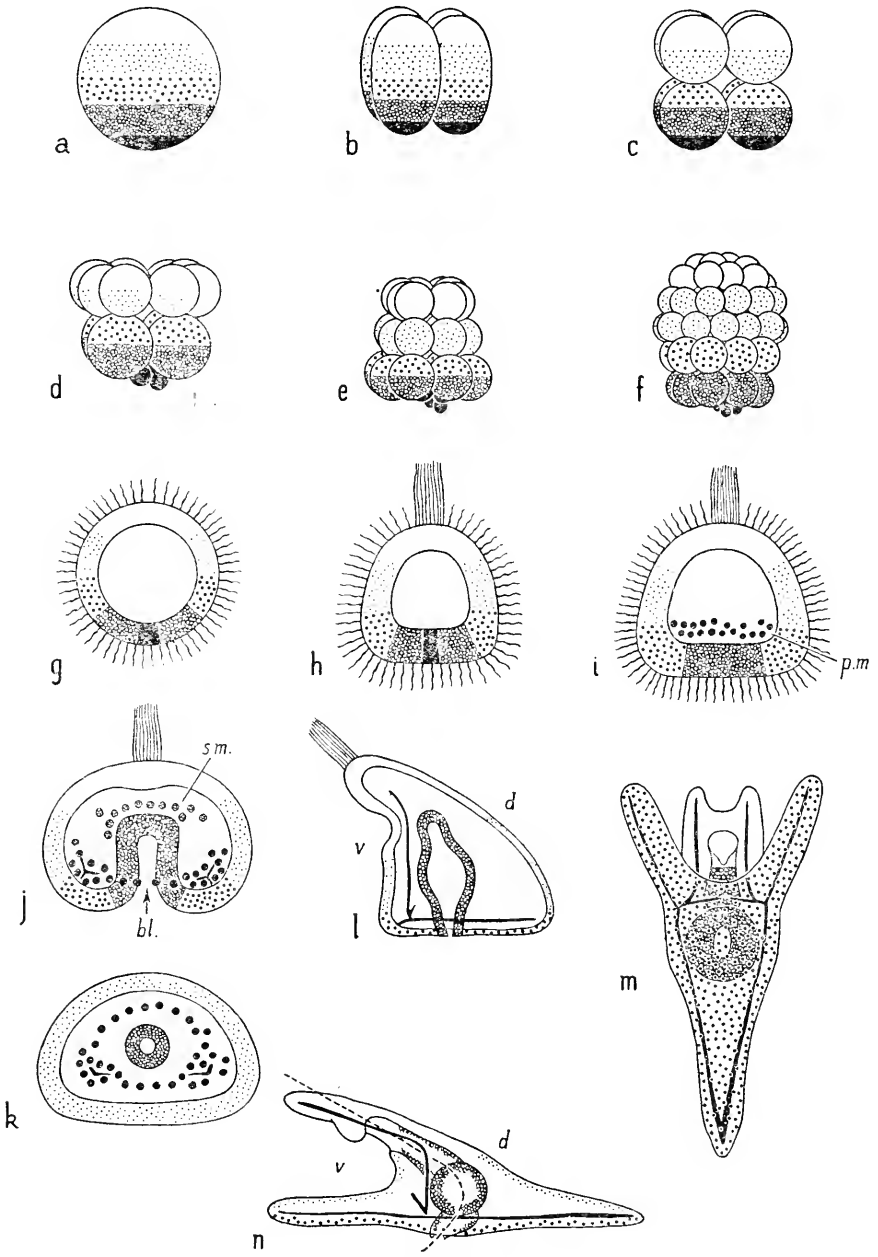

FIGURE 5.1

The development of an echinoderm. Figure *a–f* show the early cleavages stages. The shading indicates the levels which have been used in grafting

During the early cleavages, a space is gradually formed between the cells in the centre of the mass. This enlarges as the cleavage progresses, and fairly soon after the last stage mentioned above, all the cells cohere together to form a smooth hollow spherical ball with this space in the centre. This ball is the blastula, and the central space is the *blastocoel* or blastula cavity. The simple spherical shape is the most 'typical' form the blastula can take; all the many variations which we shall find in other groups can be regarded as modifications of it. In the echinoderms there are few special features to notice; but one may remark that the surface of the blastula soon becomes ciliated, and the tiny embryo escapes from its membranes, and begins an independent life soon after the cilia develop. After a short time, it grows a bunch of special long cilia (the apical tuft) at its animal pole.

Soon after the apical tuft forms, the blastula loses its strictly spherical shape and begins to flatten at the vegetative pole, diametrically opposite the tuft. This is the first sign of gastrulation, which, in essentials, consists in the folding inwards of the vegetative part of the blastula to form a pocket pressed into the cavity of the blastocoel. This pocket is the first rudiment of the gut, and is usually known as the 'primitive gut' or archenteron. Its walls make up the *endoderm*, the innermost of the three fundamental layers out of which the embryo is built. The opening by which the gut communicates with the exterior is the *blastopore*. Meanwhile, the rest of the surface of the blastula, which is not pressed inwards, forms the outermost layer or *ectoderm*. Between these, as we have seen, there should be a third or middle layer, the *mesoderm*. The main way in which the early development of the echinoderm differs from the general scheme which applies to vertebrates is that here the mesoderm originally lies, not between the ectoderm and endoderm, but right at the vegetative pole. It is formed from the micromeres and the material just above them.

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experiments; white is an-1, fine dots an-2, coarse dots veg-1, circles veg-2, and black the micromeres. Figure *g* shows a section at the blastula stage, at which time cilia develop on the surface; *h*, beginning of gastrulation and formation of apical tuft of long cilia; *i*, the micromeres come free into the blastocoel to form the primary mesoderm *p.m.*; *j*, appearance of primitive gut, with blastopore *bl*; from the tip of this secondary mesoderm (*s.m.*) is given off, the general ciliation is not shown in this or later drawings; *k*, horizontal section through same stage as *j*; *l*, flattening of ventral side, the skeleton begins to be laid down by the primary mesoderm; *m*, *n*, views of young pluteus larva from the original vegetative pole and from the side, showing the development of 'arms' from the corners of the ventral surface, and the junction of the tip of the primitive gut with the ventral ectoderm to form the mouth. The dotted line in *n* shows how the original animal vegetative axis has become bent. (After Hörstadius 1939.)

The most vegetative cells begin to break loose separately into the blastocoel even before there has been much infolding of endoderm. These are the 'primary mesoderm' cells, and they soon start secreting the first calcareous spicules of the skeleton. A similar process of shedding cells into the blastocoel goes on particularly from the tip of the primitive gut as it folds in; this is the 'secondary mesoderm', which later forms the greater part of the skeleton and muscles.

The pocket of endoderm pushed in to the blastocoel continues to elongate, producing a long finger-shaped primitive gut. The process is known as 'invagination', a term which is applied to all types of movement by which the endoderm and mesoderm are formed from the blastula; as we shall see, it covers several different sorts of foldings and cell migrations. In the echinoderms, it appears to be a simple in-pushing, like that produced when one presses a thumb into a soft hollow ball. While this is going on, the first signs of bilateral symmetry appear. The embryo, now entitled to the name of gastrula, has become somewhat conical. It begins to flatten on one side, which is the future ventral side. The elongating primitive gut turns towards the upper end of this side, fuses with it, and eventually breaks through to form an opening. This is the rudiment of the mouth; and there is now a complete tube leading from it to the blastopore, which from now on functions as the anus. The gut soon begins to differentiate into an oesophagus, a stomach and an intestine; and meanwhile four long arms grow out from the corners of the flattened ventral side, while the opposite corner of the gastrula also elongates into a single thick spike. This completes the formation of the pluteus.

2. *The gradient system*

Echinoderm eggs were some of the first with which experimentalists tried to solve the fundamental physiological problems of development. They are easily obtained in large numbers; and it is simple to free them of their membranes (by squirting them through a narrow pipette, for example). Only their small size is an impediment; but this, so long a major limitation on the kinds of experiment possible, was largely mastered by the Swedish investigators Runnström, Hörstadius and Lindahl, who combined subtle chemical methods of attack with the most delicate manipulative skill.

In the earliest experiments, which were mentioned on p. 62, Driesch showed that if the first two, or first four, blastomeres are separated by cutting along the cleavage planes, each isolated cell can form a complete well-proportioned pluteus. He concluded that these cleavages do not separate parts whose developmental fates have been already determined;

the later developmental history of a blastomere can be altered if the cell finds itself in an abnormal situation, as it does when isolated. This conclusion still stands. The cleavage pattern can be altered by various treatments, and in spite of this, normal larvae are formed. Clearly the pattern of cleavage has no decisive effect on the pattern of development.

Driesch went further in his conclusions and considered that every part of the early egg had the same potentialities. He argued that the formation of a complicated embryo from an egg all of whose parts were alike was an inconceivable achievement to be accomplished by a natural mechanism, and that its explanation demanded a non-natural agent, whose functions were to create order out of uniformity; this he called 'the entelechy'. But as a matter of fact, Driesch's basic postulate, that all parts of the egg are similar, has turned out to be untrue; and the entelechy thereby loses its main support.

The demonstration of differences within the egg has come from experiments in which the parts have been cut apart along horizonal planes. There is no need here to attempt to summarise the whole massive volume of evidence, and we will select one of the most demonstrative experiments—that in which the 32- or 64-cell stage has been dissected. Hörstadius, who did the experiment, distinguished five zones. *Animal*-1 and *animal*-2 lie at the top, and below them are *vegetative*-1 and *vegetative*-2 derived from the macromeres; at the very bottom of the egg there are the micromeres. In the first part of the experiment, each of these zones was isolated. The essential feature of the result was that each zone developed more or less in accordance with its normal fate within the embryo, only more so, if one may put it like that. For instance, *an*-1 gave a pluteus which contained only the organs appropriate to the most animal region (e.g. apical tuft, no gut); but these organs were exaggerated, in the sense that the apical tuft spread out to cover nearly the whole surface. *An*-2 also gave larvae with no gut, and usually with apical tufts, which were often enlarged (although normally *an*-2 does not participate in the formation of the tuft, which is formed from *an*-1 cells). Similarly the vegetative cells gave larvae containing vegetative organs, which again might be exaggerated. The micromeres unfortunately fail to develop anything when isollated, but *veg*-2 gives a larva with an exaggerated gut, which is often too large to fit inside the ectoderm and thus protrudes, the resulting embryo being known as an exogastrula. *Veg*-1 is highly variable; sometimes it has exaggerated vegetative organs (e.g. gut), sometimes it gives a larva consisting mainly of animal organs; in a few cases it produces a reasonably normal pluteus.

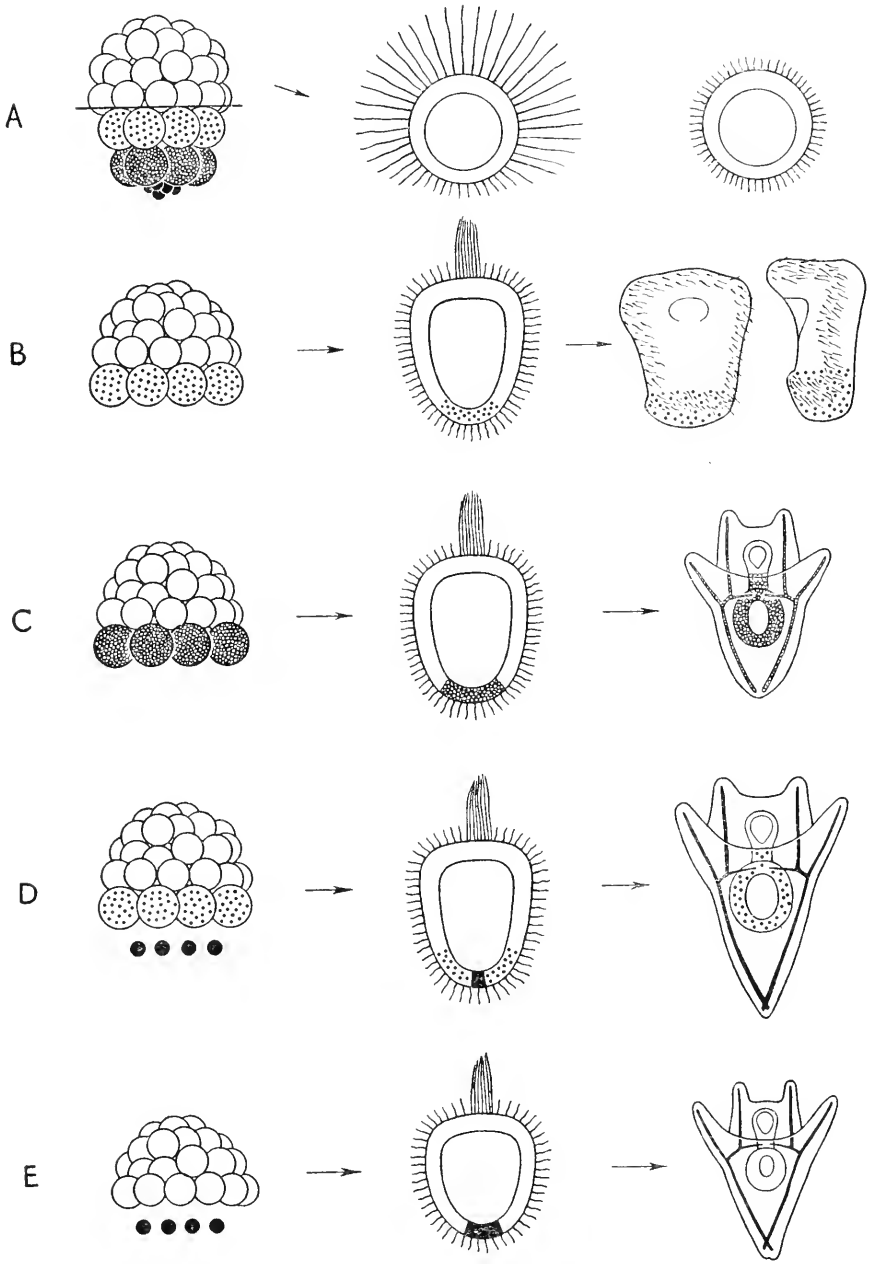

FIGURE 5.2

Combinations of the animal half with various levels along the animal-vegetative axis: *A*, the isolated animal half (an-1 plus an-2) forms a blastula nearly completely covered with apical cilia, and later a larva with no gut;

Hörstadius interpreted these results to mean that the egg contains two *gradients*, an animal one with its high point at the animal pole, and a vegetative one running in the opposite direction. Regions which are high on the animal gradient tend to form animal organs, and similarly for the vegetative. Moreover, Hörstadius proceeded to show that the two gradients interact with one another. In a very beautiful series of experiments, he combined the various layers in abnormal combinations. He found, for example, that normal plutei were formed fairly frequently when *an*-1 was combined with four micromeres, and that as the number of micromeres was reduced, so more and more 'animal' larvae appeared. *An*-2, on the other hand, required only two micromeres to produce a normal larva, and became vegetative in character if more were added. *Veg*-1 was usually swung over too much to the vegetative side by the addition of even one micromere; probably about a half would be enough to counteract its slight preponderance of animal tendencies (Cf. Fig. 5.2).

Both in the experiments in which the various zones were isolated, and in those in which micromeres were grafted, there was considerable variation in the resulting embryos. It appears that one is dealing not only with the interaction of animal and vegetative potentialities of various strengths, but that there is also another important factor, namely a tendency for the normal equilibrium between these two conditions to be restored, even when the two parts originally grafted together were not in perfect balance (see Lehmann 1945, p. 64). We will find that a similar tendency to restore a normal equilibrium condition is a very frequent influence in most developmental events. It is an aspect of *individuation* (p. 12).

There are several other types of experiments in which individuation is strikingly exhibited in the echinoderm embryo. The vegetative region, and particularly the micromeres, have a very strong ability to influence their surroundings in such a way that the latter fit into the building-up of a complete or partially complete embryo. For instance, a meridional half may be combined with an animal half in the 16-cell stage (Fig. 5.3). From this a normal pluteus will develop, the part of the animal material lying next the micromeres being, as it were, absorbed into the developmental system of the latter and converted into gut. A rather similar result is obtained if a group of micromeres is inserted among the animal cells

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B, the addition of veg-1 restricts the size of the apical tuft, but again no gut develops; *C*, with veg-2 a relatively normal embryo is formed; *D*, with veg-1 plus the micromeres, the embryo forms a gut which is abnormally large; *E*, the addition of the four micromeres to the animal half gives a nearly normal larva, although their mass is much smaller than the veg-2 material added in *C*. (After Hörstadius 1939.)

of an intact egg (Fig. 5.4). They continue developing in a vegetative way, and, further, swing some of the neighbouring animal material into line with them, so that it too develops as gut. The animal gradient of the egg is exhibited by the fact that the nearer the animal pole the graft is placed, the smaller is the gut formed.

It is important to note the similarities, and also the differences, between the production of a gut by the action of the grafted micromeres on the animal cells in the above experiments, and the 'embryonic induction' which is most strikingly seen in vertebrates. In both cases, a part of the embryo, when grafted into an abnormal situation, causes the material

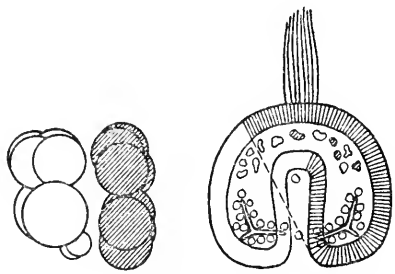

FIGURE 5.3

A meridional half of the 16-cell stage (uncoloured, on left, containing the whole an-veg axis) is combined with the animal half of the same stage (shaded). A normal embryo develops, shown in section on the right. (After Hörstadius.)

surrounding it to develop in a way which it would not normally have done. In true embryonic induction, however, the graft induces from its surroundings something of a different nature to itself; for instance, in the vertebrates, mesoderm can induce the formation of neural tissue, or in *Limnea* the gut can induce the formation of the shell-gland. In the echinoderms, the micromeres induce something which is either normally derived from micromeres or from the next most vegetative material; we are dealing with a tendency for the completion of the graft at the expense of its surroundings; moreover, these surroundings clearly play an important part in determining the nature of the structures induced. In the vertebrates, as we shall see, induction may sometimes show certain features which indicate a similar assimilative or individualising tendency, but it can also be a much more independent process, the inducer provoking the appearance of something which it does not in any way need in order to

complete itself. Thus vertebrate induction may involve both individuation and something else, which has been named 'evocation'; in the early stages of the echinoderm, we seem to have only the former.

The hypothesis of two gradients finds very strong support in the work described above. But innumerable questions immediately suggest themselves. What are these things gradients of? Are they localised in any particular part or structure of the egg? And how do they produce their

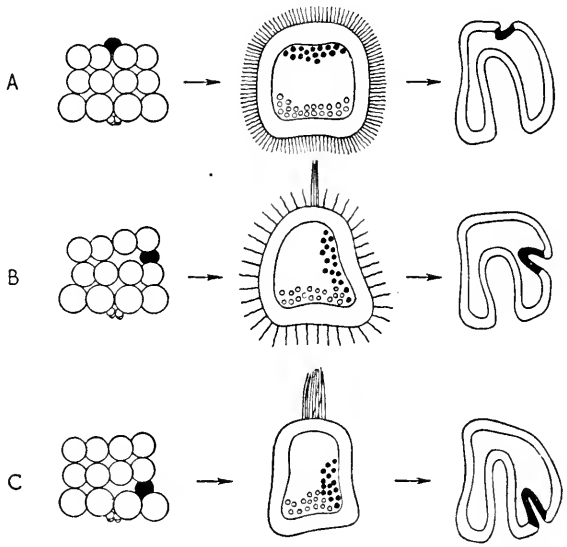

FIGURE 5.4

Grafts of the micromeres (black) at different levels along the an-veg axis. They migrate into the blastocoel cavity as primary mesoderm, and induce the formation of an archenteron from the material near them. This induced gut (indicated in black in the right-hand column) is larger the nearer the graft is to the vegetative pole. (After Hörstadius.)

effects? None of these questions can be answered with any assurance, but knowledge is increasing rapidly about them. As regards the first, we know that the gradients can be influenced by chemical substances (Lindahl 1942, Gustafson 1950). The vegetative tendencies are strengthened by the action of lithium salts. If complete eggs are treated with lithium, they form too large a gut, producing exogastrulae similar to those formed when *veg*-2 is isolated. Similarly, isolated animal halves can be brought to develop into normal larvae by lithium. The animal tendencies are increased by early treatments with thiocyanide. This, and other similar

evidence, suggests that the gradients are fundamentally based on the rates of certain critical chemical reactions. Moreover, if young embryos are constricted, by being tied in a loop of hair, the degree to which the animal and vegetative gradients interact with one another depends on the width of the connection between the two halves, which again suggests that diffusing chemical substances are involved.

We still have little idea what these substances are. Very careful measurements have failed to reveal any difference in respiration between the animal and vegetable halves. However, Child (1936) observed some years ago that by the blastula stage there is a double gradient in the rate of reduction of vital dyes such as Janus Green and methylene blue, which are indicators of redox-potential. There is a high level of reduction activity at the vegetative pole, falling off towards the animal, and simultaneously a weaker gradient running in the opposite direction from the animal pole towards the vegetative. Hörstadius (1952) has recently studied these gradients in isolated animal and vegetative halves, and in animal halves into which micromeres have been implanted, and has shown that their behaviour parallels that of the postulated gradients of animal-vegetative tendencies. The biochemical meaning of the gradients in dye reduction is not yet understood, nor can one be certain whether they are related to the causes of the animal and vegetative gradients or are merely among their effects (Fig. 5.5).

A perhaps more promising clue to the processes underlying the gradients is the fact that in rather late cleavage stages the vegetative region of the egg comes to require the presence of sulphate ions in the medium, and its development is inhibited in artificial seawaters from which they are absent. Lindahl (1942) suggests, on these and other grounds, that the reactions proceeding in the vegetative region give rise to aromatic waste products (formed from protein catabolism) which are normally disposed of by being combined with sulphate. Hörstadius and Gustafson (1954) have studied the animalising and vegetativising effects of various amino-acids as well as substances which might be expected to play a role in carbohydrate metabolism. The latter group were, on the whole, animalising, which supports the suggestion that the animal tendencies are connected with carbohydrate metabolism; but the effect of the amino-acids, although usually towards vegetativisation, was not invariably so, and further work will be necessary before the experiments can be fully interpreted.

Meanwhile there is another quite different theory in the field. Ranzi (1951) presents evidence to show that lithium and thiocyanate have important effects on the viscosity of solutions containing elongated protein

molecules, and he believes that their actions on the echinoderm egg are related rather to their influence on the physical condition of the cytoplasm than to any direct alteration of the chemical metabolism.

Finally, a very unexpected point has recently been discovered by Lindahl (1953) who has claimed that, in the two species of sea-urchin he studied, the micromeres are haploid in chromosome number. It is not clear how this remarkable example of nuclear differentiation (cf. p. 354) is related to the high 'vegetative' activity of these cells.

As regards the localisation of the gradients, most authors agree that they must be in the cortex. The argument is primarily the negative one,

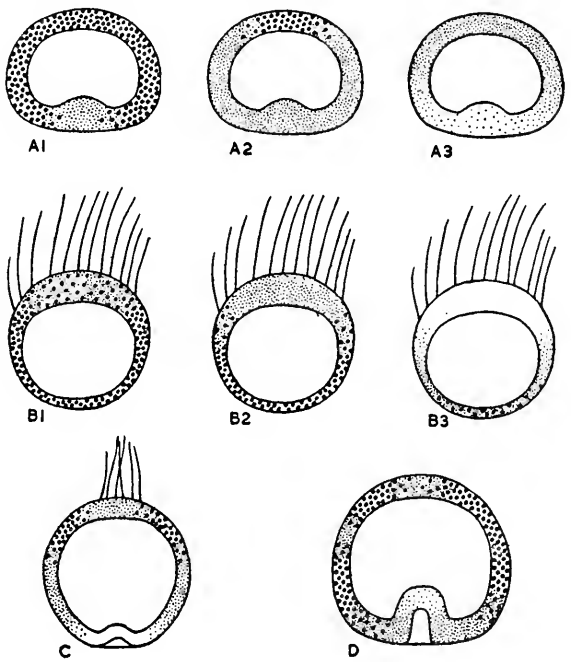

FIGURE 5.5

Sections of early gastrulae stained with Janus Green, which is at first blue (large dots) changing to red (fine dots) as it becomes reduced; in the undotted areas the colour has faded altogether. *A* 1, 2, 3, three stages over a period of 50 mins. in an isolated vegetal half, showing the reduction spreading from the most vegetative region. *B* 1, 2, 3, a period of 55 mins. in an isolated animal half, the reduction starting in the most animal region. *C*, an animal half into which micromeres have been grafted, showing the induction of an archenteron, with an associated region of rapid reduction, and the slow progress of reduction in the neighbourhood of the apical tuft. *D*, micromeres were implanted on the left between an-1 and an-2 of a normal 32-cell stage, as in Figure 5.4 middle row; a region of rapid reduction has appeared in their neighbourhood. (From Hörstadius 1952.)

that the internal regions of the egg can be shifted about by centrifugation without producing any profound result, so that the controlling gradients must be in the stiff ectoplasmic layer which is not displaced by centrifugation.

Finally, to the question of how the gradients work, we can offer at least the beginning of an answer. Although moderate centrifugation, sufficiently strong to cause considerable stratification of the cytoplasm, has no great effect on development, the situation is rather different following very intense centrifugation in a modern ultra-centrifuge. Using forces of the order of 45,000 g, on the unfertilised egg, Pease (1939) was able to throw down to the centripetal end a mass of small mitochondria-like granules. He found that if, owing to the orientation of the egg in centrifuge, these had been collected at the animal pole, the vegetative pole which lacked them was unable to gastrulate; and so, *mutatis mutandis*, was the animal pole unable to develop if all the granules had been forced to the vegetative end. He suggested that these granules are the immediate agents of differentiation.

At the present time, a great deal of attention is being paid to the behaviour of various types of cell granules in developing echinoderms. They can be roughly classified into 'mitochondria', which are larger and become sedimented at relatively low speeds of the centrifuge (giving about 16,000 g), and 'microsomes', which are only sedimented at the highest speeds (giving about 100,000 g). The mitochondria can be detected by normal cytological techniques within the cells of the embryo. It has been found (Gustafson and Lenique 1952, Lenique, Hörstadius and Gustafson 1953) that in early stages they are distributed in a gradient, decreasing in concentration from the animal to the vegetable pole (Fig. 5.6). In isolated animal or vegetative halves, and in halves which have been 'animalised' or 'vegetalised', the gradients become altered in a manner exactly parallel to that of the basic animal and vegetative gradients, or the gradients in dye reduction mentioned above. This makes it most probable, then, that the mitochondria are rather directly concerned with the fundamental developmental processes. Gustafson (1953, 1954) has made extensive studies on their metabolism, and suggests that they are connected with the synthesis of the fibrous proteins of the apical tuft, which is the most characteristic animal organ.

The mitochondria can be formed anew during development. Harvey (1946) centrifuged echinoderm eggs in a medium which had a gradient of specific gravity, and showed that under these circumstances, the egg may be split into several fragments, each containing the constituents of a particular density. Fragments made in this way may develop normally

even if they originally contain no mitochondria; but it is found that mitochondria gradually appear. It seems probable that they are formed from the cytoplasmic particles of smaller size (the microsomes). Hultin (1953*a*, *b*) has summarised a number of studies in which radioactive isotopes have been used to follow the processes of protein synthesis in the cell as a whole and in the various groups of particles (see also Kavanau 1953). During the early cleavage stages, it is in the microsomes that this

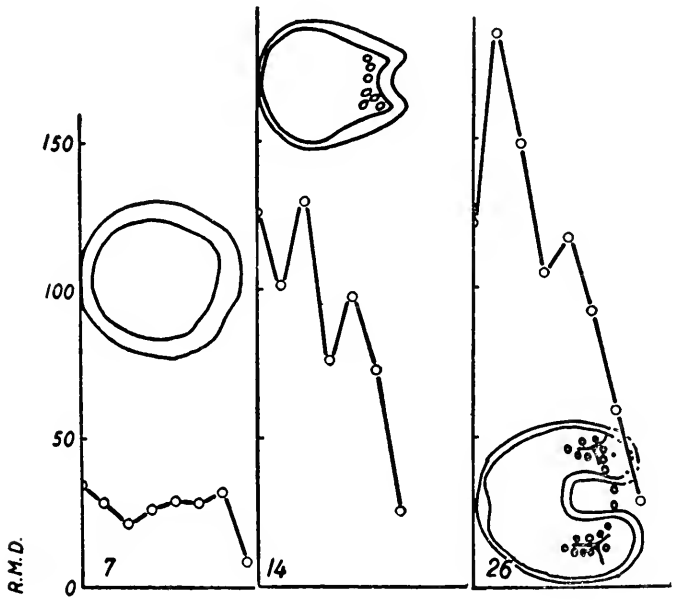

FIGURE 5.6

Graphs showing the relative density of the mitochondrial population (R.M.D., plotted vertically) at various levels of the animal-vegetative axis at 7, 14 and 26 hours of development (blastula to mid-gastrula). (From Gustafson 1954, after Lenicque, Hörstadius and Gustafson 1953.)

synthesis is proceeding most rapidly, but by the early gastrula the mitochondria are becoming very active. Hultin suggests that the mitochondria are built up either from, or at least by the influence of, the microsomes. The reactions between these two types of particles are, however, probably reciprocal, since there is reason to believe that, once the mitochondria have been formed, they induce a high rate of activity in the microsomes, and that it is actually at these small particles that protein synthesis proceeds most rapidly. The evidence from work with isotopes suggests that this synthesis begins to get fully under way at about the time of gastrulation.

Perlmann (1953) has indeed been able to detect, immunologically, the appearance of at least one new antigenic substance (protein?) at that time.

The study of the roles of the various types of cytoplasmic particle has only begun very recently and there is still much to do. Hultin emphasises a point which is probably of considerable general importance, namely that we must think of the developing cell as containing populations of mitochondria and microsomes which are continually reacting on and influencing each other; and one may add, of course, that the nucleus with its contained genes must be also involved in the same general network of cause and effect.

All the grafting experiments on the gradient system described above were carried out on the early cleavage stages, before the embryo has sixty-four cells. Isolations of animal and vegetative halves can be made at later stages, and it is found that the divergence of the isolate from its normal fate remains much the same until the early blastula stage (about ten hours after fertilisation). From that time onwards, the developmental fate of the regions rapidly becomes more fixed, so that, for instance, an animal half isolated from a later stage develops only the normal sized tuft of cilia, and a vegetative half only a normal gut. The developmental fate is not entirely determined in such halves, since if micromeres are implanted into animal halves isolated at various times, it is found that they can induce some vegetativisation even in halves which, if left isolated, would develop only into their original presumptive fate. The embryonic materials are, however, rapidly losing their lability, and by about sixteen hours after fertilisation, an animal half can no longer be affected by implanted micromeres. It is interesting to note that if an animal half is isolated at the 32-cell stage, and allowed to develop in isolation for some time before the micromeres are grafted into it, it loses its reactivity earlier than it would do as part of a whole egg; presumably its isolation from its vegetative partner allows its animal tendencies to become fixed earlier.

3. *The dorso-ventral axis*

It was stated earlier (p. 48) that the determination of a dorso-ventral axis, which confers a bilateral symmetry on the egg, is one of the fundamental steps in development. It may well be asked how, and at what time, this step is taken in echinoderm development. As a matter of fact, the evidence suggests that there is some trace of bilaterality even in the unfertilised egg, presumably dependent on factors operating during the maturation of the egg in the ovary. If such eggs, or early cleavage stages,

are sectioned vertically, there are slight differences in development according to the plane of the section. These can be best interpreted by the hypothesis that one side already has a slight tendency to become ventral, and usually does so. If a ventral half is separated from a dorsal half, the ventral face appears in its original position in the former, but in the dorsal half, the axis may be reversed, so that the eventual ventral side of this half-embryo appears on the side which, in the undisturbed egg, would have been dorsal.

This original organisation is only a labile one, and can be overcome by a number of different influences. It becomes determined at about eight hours after fertilisation. If eggs are strongly stretched before this, by being sucked into a tube with a narrow lumen, the dorso-ventral axis is altered so as to run along the length of the elongated egg. Strong staining of one end of such an egg with Nile Blue sulphate will cause this end to become dorsal; perhaps it would be better to say that it will cause the other end to become ventral, since it is probable that the ventral side plays the lead in the development of the axis, and that it is a suppressive action of over-staining which is the operative mechanism in the experiment. Many other chemical substances can influence the determination of the axis (see Review in Lehmann 1945). Gustafson and Sävhagen (1949) have recently shown that weak solutions of detergents will suppress the development of the oral or ventral side entirely, so that radially symmetrical larvae are formed. A similar result was produced by Hörstadius and Gustafson (1954) who treated the eggs shortly after fertilisation with antagonistic analogues (or 'anti-metabolites') of certain vitamins, etc. They suggested indeed that these substances were actually operating as detergents rather than by inhibiting the growth-promoting properties of the vitamins.

SUGGESTED READING

Hörstadius 1939, 1949, Gustafson and Lenique 1952 or Gustafson 1953, 1954, Hultin 1953*b*.

CHAPTER VI

SPIRALLY CLEAVING EGGS

THE SPIRAL type of cleavage is sufficiently definite to allow one to recognise the existence of a common pattern in several groups of animals which are not otherwise very closely related (molluscs except cephalopods, nemerteans, platyhelminths, annelids); but overlying the basic similarities there are very many variations in detail, both in the early cleavages and in the types of larvae which are produced by early development. To keep within the limits of space available here, it will be necessary to attempt a ruthless simplification.

The cleavage begins by two more or less vertical divisions, cutting the egg into four blastomeres, which are typically somewhat twisted in relation to each other. These four are conventionally known as *A*, *B*, *C*, and *D*. In the next few cleavages, each of these cells remains as a fairly large macromere, and gives off a succession of smaller micromeres, the first group of which are known as 1*a*, 1*b*, 1*c*, 1*d*, the second group as 2*a*, 2*b*, 2*c*, 2*d* and so on. The cleavage spindles by which these divisions occur do not lie either vertically or horizontally, but at some angle between; and if the spindles are tilted to the left of the vertical in the formation of the first group, they will be tilted to the right for the next group. There is in fact an alternation from one tilt to the other. While the macromeres are giving off new rings of micromeres in this way, the already formed micromeres continue to divide in the normal way, again with their spindles tilted like those of the macromeres (Fig. 4.2, p. 61).

This regular pattern usually continues for four division-cycles. The subsequent fate of each of these cells has been followed in detail, and we know exactly what organs each will form in the later embryo. There is no need to go into great detail about this here. Roughly, the macromeres form endoderm, and most of the micromeres ectoderm; but there are two special micromeres which produce the mesoderm, and which we shall find play a peculiarly important role in the mechanics of development. These are the cells 2*d* and 4*d*, both of which, as their designation indicates, are ultimately derived from the *D* macromere.

Events after the initial cleavage vary a great deal in different groups. In many species a larva is formed; and this is usually some variety of the 'trochophore' type (Fig. 6.1). In the development of this, the ectodermal micromeres grow down over the macromeres, which bend inwards to form

a pocket-shaped primitive gut, which gradually elongates until the whole embryo assumes a shape like an old-fashioned peg top. There is usually an apical tuft of cilia, and a strong ciliated band (the prototroch) more or less at the equator. Meanwhile the 4*d* mesoderm cell has been covered over by ectoderm, lying in the corner at the boundary between ectoderm and endoderm. It divides into two, and each of these daughter-cells gives off a series of endomesoderm cells. In molluscs, these again become scattered, but in annelids they remain joined together as two long bands.

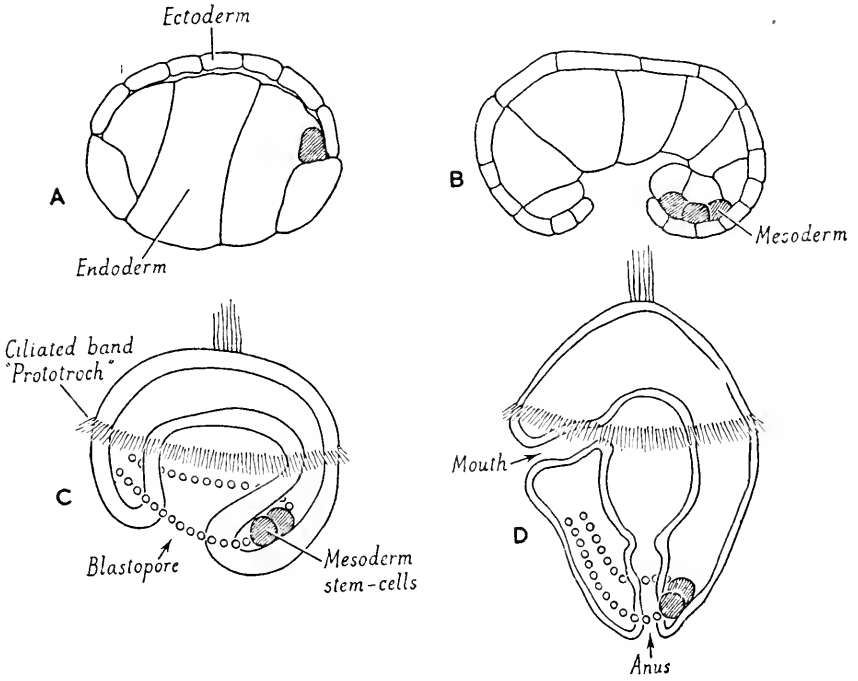

FIGURE 6.1

Gastrulation and formation of the trochophore larva in spirally cleaving eggs. *A* shows a section through a late cleavage stage, the micromeres lying directly on top of the larger macromeres, the slight space between them corresponding to the cavity of the blastula. Some cells from 2*d* and 4*d* (shaded) have sunk beneath the surface. *B*, the micromeres spread over the macromeres, which are being drawn up into the embryo, forming the beginning of the primitive gut. *C* is an early trochophore; note the two large cells, derived from 4*d*, each of which is budding off a row of mesoderm cells which extends round the blastopore between the ectoderm and endoderm. *D*, the trochophore is beginning to elongate, and the mesoderm bands are swung into a vertical position (this is typical of certain worm embryos, such as those of *Polychaetes*). A mouth has appeared, either as a new opening where the primitive gut has broken through the ectoderm, or in some forms by the blastopore becoming constricted into two.

These at first extend round the gut which is being pushed in from the blastopore, but as the whole embryo elongates, the mesoderm bands come to lie more or less vertically. Finally a mouth is formed, either by the tip end of the gut breaking through the ectoderm to the exterior, or in some forms by a constriction which divides the blastopore into two. A trochophore larva of this kind is obviously only a minor variation from the general structure of a gastrula, and the whole egg takes part in its formation.

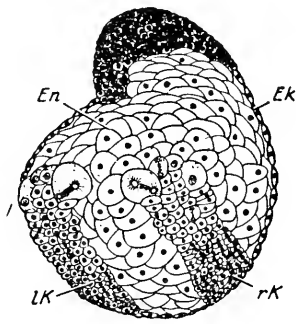

FIGURE 6.2

Direct development (without larval stage) in a spirally cleaving egg (the freshwater oligochaete *Tubifex*). The macromeres have cleaved to give a compact mass of largish endoderm cells (*En*), while most of the micromeres have formed small ectoderm cells lying on the other side of the egg (*Ek*). The cell *2d* has, however, produced two groups of 'stem-cells', each comprising one neuroblast and three secondary myoblasts; these bud off a row of neural cells and three rows of ectodermal muscle cells. Below each of these columns of four are mesoderm-stem cells derived from *4d*, which produce the accompanying mesoderm. Each column is known as a half-germ-band (*rk* and *lk*); they unite on the other side of the embryo to form the head, and, as they grow, to give rise to the rest of the body. (After Penners.)

In these forms with larval stages in their development, the formation of the adult does not take place till considerably later; we shall not discuss the processes by which this eventually occurs except to point out that the ectoderm and mesoderm of the adult ultimately trace back to *2d* and *4d*. In some spirally cleaving types, such as oligochaetes, the development of the egg is 'direct', in that the adult is formed without passing through any special larval stage. In these animals it is difficult to find anything which can be called a normal gastrula stage. The 'somatoblasts' *2d* and *4d* each divide into a right and a left half; and the cells so formed become the mother-cells from which a long string of daughter-cells is budded off.

The cells derived from *2d* form ectoderm, and overlie those from *4d* which form mesoderm. Thus two bands are produced, one on the right and one on the left, both lying on the surface of a mass of cells derived from the *A*, *B* and *C* quadrants, and each consisting of a core of mesoderm covered by ectoderm. The mother-cells continue to divide, and as these bands elongate, they push out over the surface until they meet and fuse; from the fused bands the adult worm develops. Thus in these eggs, the whole embryonic portion is formed from *2d* and *4d*, the rest of the cells taking little part.

A very large number of experiments have been made on spirally cleaving eggs, but rather little insight has been gained into the factors which control their development. In general, it is found that from a very early stage isolated blastomeres behave as though they were still part of a complete egg, and develop only into those organs which they would have formed if left undisturbed (Review: Schleip 1929). This fact gave rise to the suggestion that there is, in these eggs, a strict localisation of 'organ-forming areas', or regions each of which could develop only into certain definite organs. The egg was considered to be a mosaic of such areas, and such 'mosaic' development was contrasted with the 'regulation' development found for instance in echinoderms.

We now know that the mosaic character is by no means absolute. As an example which demonstrates both the truth and the falsity of the idea, we may consider the egg of the nemertean *Cerebratulus*. Hörstadius (1937) has made some isolations and recombinations of the various rings of cells at the 16-cell stage, quite comparable to his experiments on echinoderms described on p. 85. In *Cerebratulus* he found completely 'mosaic' behaviour; each ring of cells, when isolated, formed only what would be expected of it, and in combinations there was no sign of interaction between the animal and vegetative groups. But if one goes back to a slightly earlier stage, things are not quite the same. Any nucleated fragment cut off from the unfertilised egg, can, after fertilisation, develop into a normal embryo. And even though the differences along the animal-vegetative axis are fixed as early as the 8-cell stage, those in the other plane are certainly labile as late as the four cell, since the isolated first four blastomeres may each give a normal larva. Thus there must be some process of determination which gradually fixes the manner in which the parts of the egg can develop; but, since this is complete by the 8-cell stage, it must go much faster than in echinoderms, where we saw that regulation is possible considerably later; and unfortunately in the mosaic eggs we know of nothing which controls development in a way comparable with the animal and vegetative gradients (Fig. 6.3).

In many of the spirally cleaving eggs, the determination of future development is complete (or nearly complete) even earlier than in *Cerebratulus*. We have seen that the 2*d* and 4*d* cells are particularly important especially in species with no larval stage; and it is often found that the *D* quadrant is already different from the others from the very

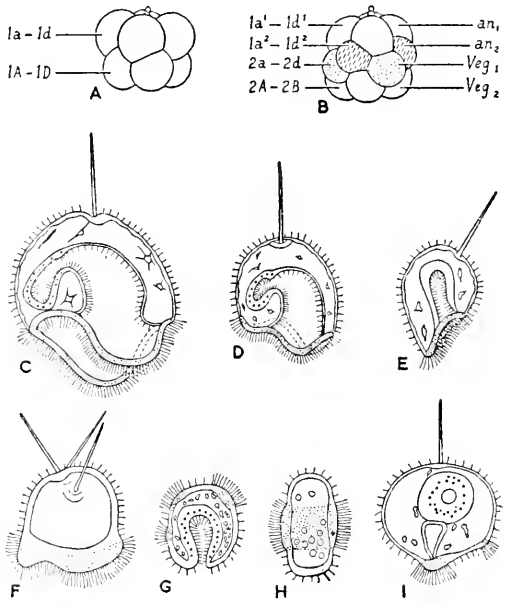

FIGURE 6.3

Mosaic development in the nemertean worm *Cerebratulus*. *A* and *B* show the normal 8- and 16-cell stages. *C* is the normal 'pilidium' larva (a variety of the usual trochophore larva). *D* is a relatively normal pilidium from an isolated blastomere from the 2-cell stage. *E*, the same from an isolated blastomere of the 4-cell stage. *F*, larva from isolated four animal cells from 8-cell stage; note the absence of any gut and the exaggeration of the apical tuft. *G*, larva from four vegetative cells isolated at 8-cell stage. *H*, middle two layers (an_2 and veg_1) isolated from 16-cell stage. *I*, larva from combined an_1 and veg_2 from 16-cell stage. Note the failure of regulation in *H* or of interaction in *I*. (From Hörstadius 1937.)

beginning of development. Thus in the oligochaete *Tubifex* (Review: Lehmann 1948*a*) only the cell containing the *D* quadrant will develop any embryonic structures if the first two or the first four blastomeres are isolated. There is therefore already something in the *D* quadrant which is necessary for the formation of an embryo. But this does not mean that all the details of development have been completely fixed by this time.

Perfectly normal embryos are formed if *A*, *B* or *C* quadrants are eliminated, and this necessitates some replacement of them by converting part of the *D* substance for the purpose. Similarly the macromere 4*D* can be dispensed with. Even if the individual somatoblasts are killed at the stage when the germ-bands are beginning to form, some regulation is still possible. For instance, if the mother-cells of the two ectoderm bands are killed, the embryo has at first no ectoderm, but some is later formed by a conversion of mesoderm cells. If the mesoderm mother cells are killed the mesoderm is not produced from any other element; but it is found that there are characteristic defects in the development of the uninjured ectoderm, which points to the existence of essential influences of mesoderm on ectoderm in normal development (Penners 1938). Thus the mesoderm seems to have in some sense a leading role in the whole embryogenesis; we shall find a much more striking example of the same thing in vertebrates.

The essential substance of the *D* quadrant can sometimes be seen, in the form of a special type of cytoplasm. In the mollusc *Dentalium*, for instance, the cleavage is very oddly modified in connection with a region of clear cytoplasm lying near the vegetative pole. Before the first division, this material is pushed out from the egg in a broad pseudopodium-like lobe. The cleavage plane runs in such a way that the whole of this gets into one of the two daughter-cells; when the lobe has been retracted and the whole cell rounded up, this blastomere is considerably larger than the other. A similar process occurs in the succeeding division, and most of the material of the lobe eventually gets into the *D* blastomere (some may be included in *C*). One of the early and classical experiments on spiral eggs is that of E. B. Wilson, who showed that when the polar lobe is removed the development of the whole embryonic region (derived from 2*d* and 4*d*) is suppressed (Fig. 6.4). Perhaps more surprising is a recent result (Novikov 1940, on *Sabellaria*); by treatment with KCl, the first cleavage can be made to become equal, so that the polar lobe substance get into both the first two blastomeres instead of only into one. Twin embryos are formed. This must involve a considerable amount of regulation, so here the lobe material has acted, not merely as a region of the egg whose fate has been determined precociously, but as one which can initiate embryonic development by the cells surrounding it. Other agents (e.g. abnormal temperatures, anaerobiosis, etc.), may upset the position of the first cleavage spindle in many types of spirally cleaving eggs, with the result that twins are produced (Tyler 1930).

We therefore seem to be confronted with the situation that, in the so-called mosaic eggs, there is at a very early stage (which varies from

before fertilisation till the time of the second or third cleavage) a localisation of types of cytoplasm which control the direction of later development; but one of these substances, namely that which normally gets into the *D* quadrant, can in some species cause a considerable amount of reorganisation to go on in its neighbourhood, so that in eggs of this type a whole embryo tends to be formed around it.

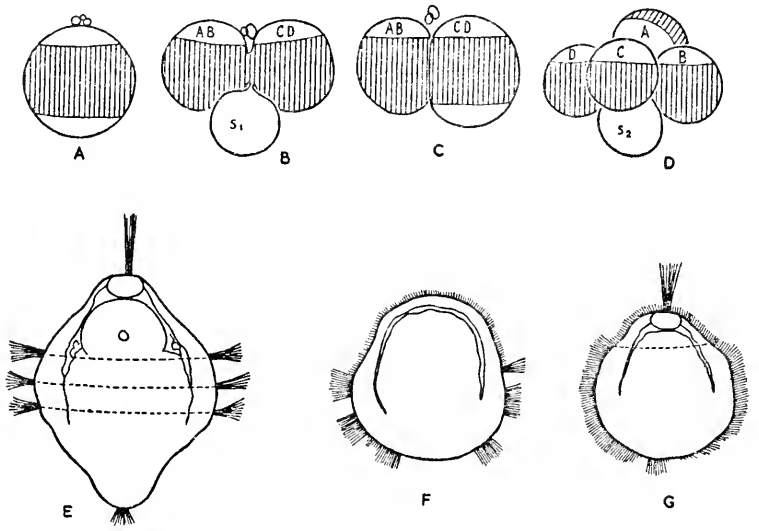

FIGURE 6.4

The polar lobe in the mollusc *Dentalium*. In the uncleaved egg (*A*) there are specialised oöplasms at both animal and vegetable pole. At the first division (*B*), the vegetative pole plasm is protruded as a 'first polar lobe' (S_1). After the division this is withdrawn into the *CD* blastomere, whence it protrudes again as a 'second polar lobe' (S_2) at the second division *D*, after which it is withdrawn once more into the *D* blastomere. At *E* is shown the normal trochophore larva; at *F* and *G* the defective larvae produced when the first (*F*) or the second (*G*) polar lobe is removed. (After Wilson.)

Recent investigations have tended to concentrate on two problems; to discover more about the nature of the substances whose localisations are responsible for the mosaic aspects of development in these forms, and to try to find out the factors which bring about the localisation. Some progress has been made in both directions.

Local differences in the cytoplasm, which are invisible in normal life, can sometimes be revealed by treatment with suitable dyes or histochemical reagents. Vital dyes which act as pH or rH indicators (such as

Janus Green, Neutral Red, etc.) often show different colours in the different regions of mosaic eggs; usually these differences are related to the main animal-vegetative axis. In typical regulation eggs, such as echinoderms, the differences are absent or at least much less well developed, but the same is also true in some mosaic eggs, where they would be expected. It is not easy to give an exact interpretation of the phenomena, since it is notorious that when indicator dyes are in the presence of protein, their behaviour is atypical, and one cannot simply deduce the pH or rH from the colours which they take up. Nevertheless, the existence of differences in colour certainly demonstrates the presence of some differences or other in the cytoplasm, even if it is unsafe to conclude much as to their nature. Histochemical tests for various enzymes (e.g. indophenol-oxidase), or fixed –SH groups, ascorbic acid, etc., have also revealed certain cases in which these substances are strictly localised within mosaic eggs. The subject has recently been reviewed by Needham (1942, p. 131 *seq.*) and Brachet (1944, p. 271, *seq.*). It will be seen from their discussions that the biochemical interpretation of the findings is not clear in this instance also. In particular, the suggestion of Ries (1942), who has been one of the most active workers in this field, that the most important differences between the regions of mosaic eggs are related to the intensities of respiration, is almost certainly based on too optimistic a neglect of the possible sources of uncertainty. Nevertheless it is important that a beginning at least has been made with the biochemical recognition of cytoplasmic localisations.

It has also been possible to obtain some information about the physical properties of the substances involved in the mosaic regions. One may ask whether they are small-molecular substances, such as amino-acids or vitamins; or on the other hand are they larger entities which should be regarded as cell constituents rather than chemical molecules? The evidence which has been available for some time, that they can be shifted about within the egg-cell by centrifugation, provides grounds for preferring the second alternative. Recently Lehmann and Wahli (1954) have made a careful study with the electron microscope of the structure of the cytoplasm in the various blastomeres of *Tubifex*. They find that at a fairly early stage there are characteristic quantitative differences between the cells. Thus by the time the four sets of micromeres have appeared, the cell 2*d* (which will produce the embryonic ectoderm) has more of the basophilic fibrillar cytoplasm, with many small globular particles and peculiar spindle-shaped bodies, whereas 4*d* (which gives the embryonic mesoderm) has sparse fibrillar cytoplasm, few of the globular or spindle-shaped bodies but many of a larger type of particle which can be designated

as 'mitochondria'. These differences, which it must be remembered affect only the concentration of the particles, none of which are completely absent in any region, cannot be seen in the newly fertilised egg, which contains a population of microsomes which appears uniform in the electron microscope. It seems most probable, however, that these microsomes differ in their chemical properties, and that it is the microsome population which determines the character of the ooplasms.

The second problem which has been attracting attention recently is that of the mechanisms of localisation. The original distribution of materials in the egg can be fairly easily disturbed by centrifugation, as a consequence of which the egg contents become stratified into layers of different specific gravity. As would be expected if the eggs behaved in a strictly mosaic manner, this stratification frequently leads to the production of abnormal embryos. But this is not always the case; almost perfectly normal larvae may develop from eggs which have suffered a severe stratification. It used to be thought that the explanation of this must be that the substances which become stratified are not those which are morphogenetically active, but comprise only relatively neutral materials such as yolk. However, the application of histochemical tests has demonstrated the presence of several important enzymes in the stratified layers, and it therefore becomes rather unplausible to advance this hypothesis. Raven (1948) has made a particularly careful study of the phenomena in the snail *Limnea*. He showed that in the unfertilised egg there is a visibly differentiated 'sub-cortical plasm' located near the vegetative pole, and that soon after fertilisation an 'animal plasm' appears near the animal pole. These two plasms move in definite ways during the early stages of development; before the first cleavage, the sub-cortical plasm spreads upwards so as to clothe the entire surface of the egg just below the cortex, while the animal plasm also extends somewhat, but eventually comes to lie mainly in the micromeres (Fig. 3.3, p. 49). If an egg is centrifuged in such an orientation that the pole plasms are moved away from their normal location, it is found that they very soon make their appearance again in their original positions. The rapidity of this distribution differs according to the exact stage when the centrifuging is carried out, since the viscosity of the cytoplasm varies throughout the progress of the cleavage. Raven claims that the redistribution of most substances can continue, even after the appearance of the first few cleavage faces; only the protein yolk appears to be relatively immobile and unable to pass through the cell walls.

There must therefore be some general condition which controls the disposition of the various regions of egg cytoplasm. Both Raven, and

Lehmann (1948*a*,), who has described very similar phenomena in the oligochaete *Tubifex*, believe that this control is exerted by the cortex. They suggest that the polar regions of the cortex are not disturbed by centrifugation, and that they exert specific attractions on the materials which should form the appropriate pole plasms, so that these cytoplasmic localisations can become reconstituted. This suggestion would tend to suggest similarities between the mosaic spiral-cleaving eggs and the typical regulation eggs of the echinoderms, in which we have seen that the cortex is probably the seat of the epigenetic gradients which play the main part in early development in those forms. It might be, indeed, that in the spiral eggs also the difference between the animal and vegetative cortical regions is graded, as it is in the echinoderms, but that in the former this cortical gradient is very rapidly converted into a qualitative distinction by the attraction of definitely different materials to form the two pole plasms.

Although an attraction between cortical regions and particular types of cytoplasm may be of major importance in controlling localisation in mosaic eggs, it seems doubtful whether it can be the whole story. The restoration of normality in a centrifuged egg is not merely a matter of attracting one specific substance to each end of the original axis, but of redistributing the whole set of substances which have been deranged. Costello (1948), another recent worker in this field, argues that there must be a more generally pervasive system of relations which orders the egg cytoplasm throughout its mass. He refers to this as 'ooplasmic segregation' and has advanced an ingenious hypothesis as to its nature, based on the idea of diffusion gradients. It seems not impossible that his ideas and those of Raven and Lehmann will eventually come together; perhaps the polar cortical regions establish the initial difference between the two ends of the main axis, and this is transmitted through the mass of the egg by some diffusion mechanism similar to that which Costello has indicated.

We have already seen that the various regions which become localised in so-called mosaic eggs are not quite so rigidly determined in their developmental fate as the word 'mosaic' originally implied. It is also important to realise that in these eggs other mechanisms are at work, which are quite different from the localisation of distinct types of cytoplasm. Raven (1952) has made a particular study of them in *Limnea*. He found that treatment with lithium, at stages earlier than the twenty-four cell, caused a reduction in the head. The medio-dorsal part was most strongly affected, and the effects show a series of grades leading to complete disappearance of this region and fusion of the eyes. This is a typical example of the type of behaviour spoken of as a 'field' process; that is

to say, the whole region is behaving as in some sense a unit within which some property, which controls future development, is distributed in a graded manner. When the region is affected by some substance toxic to it (lithium in this instance) it reacts as a whole, every part as it were sinking in grade, so that the more central parts take on the character normally proper to more peripheral regions.

Again in *Limnea*, Raven has demonstrated the importance of typical processes of induction. Normally a shell-gland is formed by the ectoderm, in the area where the gut comes into intimate contact with it. Raven was able to induce the blastula to exogastrulate, and showed that if the gut did not touch the ectoderm, no shell-gland was formed. Moreover, in cases of abnormal gastrulation, in which the gut had reached an atypical part of the ectoderm, a shell-gland was formed in this unusual position. Thus there is little doubt that the shell-gland is induced by contact between the gut and the ectoderm. We shall see that such processes play a major role in the development of vertebrates. In the mosaic eggs, although there are probably many more of them than have yet been discovered, they seem to be of secondary importance in comparison with the process of cytoplasm localisation (Fig. 6.5).

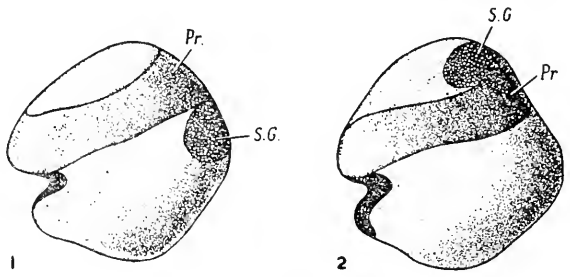

FIGURE 6.5

Induction of the shell gland by the archenteron in *Limnea*.

Normally the shell gland (*S.G.*) lies posterior to the main band of cilia (Prototroch, *Pr.*), where the archenteron comes in contact with the ectoderm (1). But if, owing to abnormal gastrulation, the archenteron reaches forward to the pre-trochal ectoderm, the shell gland is formed in the corresponding place, (2). (After Raven 1952.)

SUGGESTED READING

Hörstadius 1937, Raven 1948, Lehmann 1948*a*, Costello 1948.

CHAPTER VII

THE ASCIDIANS AND *AMPHIOXUS*

THE ASCIDIANS stand midway between the invertebrate and vertebrate kingdoms. In the early development of many of them, a tadpole-like larva is formed, which is furnished with an axial notochord with an accompanying dorsal nerve-cord. These formations disappear or become greatly modified in the final metamorphosed adult, but in so far as the animal possesses them at all, it is to that extent entitled to be reckoned as a member of the chordates. It is more doubtful whether the ascidians should be regarded as exceedingly primitive members of the group in which the larva foreshadows the later evolutionary history, or as a specialised group of degenerate forms in which the chordate larva is the only remaining sign of a more glorious evolutionary past. Perhaps both views have something of the truth; the ascidians may represent a chordate stock which began to degenerate after only a short history of progressive evolution. In favour of this interpretation is the fact that in the very early development of the egg, which leads to the formation of the chordate larvae, they show many of the features which one would expect in a primitive, as opposed to a highly evolved, member of the Chordata. The eggs, in fact, bear a very striking resemblance to those of *Amphioxus*, which is undoubtedly a very primitive chordate. It will, indeed, be convenient to treat the early development of ascidians and *Amphioxus* together. The simplicity and clarity of their developmental processes, together with the primitive position of *Amphioxus* in the evolutionary scheme, has for long rendered these animals classical embryological material.

The first full study of the *Amphioxus* egg was made by Hatschek in 1881. Unfortunately many of the authors who followed him (Wilson, MacBride, Cerfontaine) misinterpreted the orientation of the early embryo, thinking that the anterior was the posterior and vice versa; and some textbooks still follow their erroneous accounts. The true state of affairs was made out by the American embryologist Conklin (1932). In the earlier years of the century, the same author had laid the foundations of our knowledge of ascidian experimental embryology (Conklin 1905).

In both *Amphioxus* and the ascidians, the egg is fairly small (about 0·1 to 0·2 mm. in diameter) and contains only a moderate amount of yolk. Before fertilisation, the egg nucleus is in the form of a large germinal

vesicle, with a diameter up to half that of the egg. It lies at one side, closely against a peripheral layer of clear cytoplasm which encloses the main bulk of the egg. Conklin points out that in *Amphioxus*, and probably in ascidians, the attachment of the egg to the wall of the ovary is by the end containing the germinal vesicle, that is by the animal pole, whereas in most invertebrates it is the vegetative pole which is attached; this difference he correlates with the fact that the vertebrates have a dorsal nerve cord and the invertebrates a ventral one, so that the two kingdoms seem to differ by a reversal of their dorso-ventral axis. The suggestion is an interesting one, but it is by no means clear that most vertebrate eggs are like *Amphioxus* in having their animal pole attached to the ovary wall. In the frog, the point of attachment seems to be slightly below the equator.

Both ascidians and *Amphioxus* provide beautiful examples of the important fact that an egg is not a mere lump of featureless cytoplasm furnished with a haploid nucleus, but on the contrary has an effective architecture of its own. The eggs of some ascidians, such as *Styela*, described by Conklin, are perhaps the clearest instances of this to be found in the animal kingdom, since in this form several different regions of cytoplasm can be visibly distinguished owing to their content of coloured granules, mitochondria, yolk, etc. Before fertilisation, the egg of *Styela* has a peripheral layer of clear yellowish cytoplasm, inside which is a grey yolk-laden cytoplasm, and the large germinal vesicle filled with clear sap (Fig. 7.1). The sperm enters always near the vegetative pole. As it penetrates it sets off a reaction of the egg surface, which in this instance involves a streaming of the yellow peripheral cytoplasm downwards to the vegetative pole. Simultaneously the germinal vesicle undergoes the reduction divisions and breaks down to give rise to the polar bodies; the clear plasm thus released also moves down the egg and lies above the yellow material just above the vegetative region. The sperm head now starts to move upwards, remaining fairly near the egg surface. As it does so, it appears to pull the two layers of clear and yellowish cytoplasm along with it, the yellowish material remaining on the surface, while the colourless extends deep into the centre of the egg. After throwing off the polar bodies, the egg nucleus descends towards the sperm nucleus, and they meet just below the equator but away to the side of the egg towards which the sperm has been travelling. Since the yellow and colourless materials have been accompanying the sperm, they are now seen as two crescents, clear above and yellow below, with their thickest part in the meridian along which the sperm moved. This meridian is clearly a plane of bilateral symmetry in the egg. The first cleavage plane falls along it, and in the final embryo it is the plane dividing the right side from the

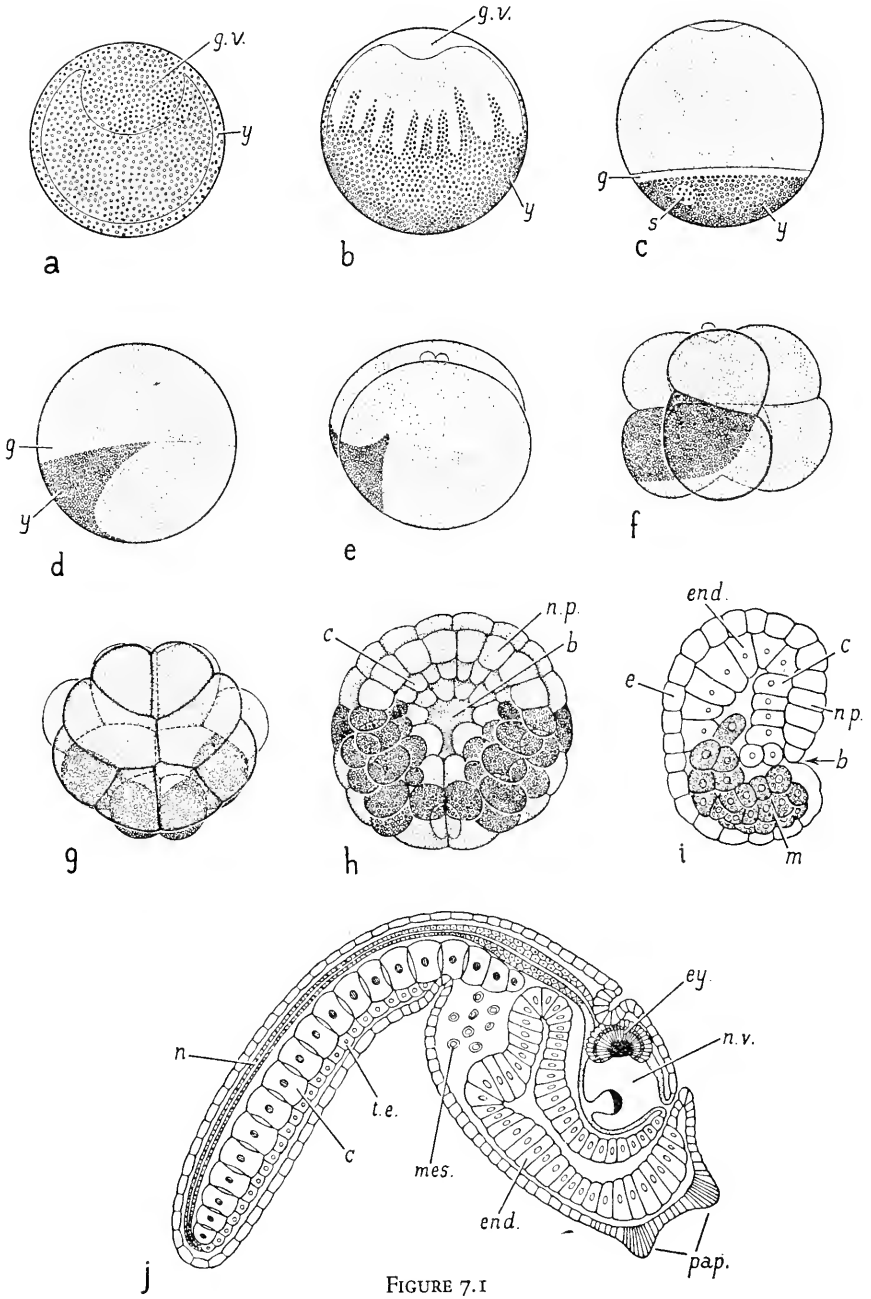

FIGURE 7.1

The development of the Ascidian Styela.

(*a*) Before fertilisation, showing the large germinal vesicle (*g.v.*) and the peripheral yellowish layer (*y*), and the inner grey yolky cytoplasm.

(*b*) Immediately after fertilisation. The germinal vesicle has ruptured and

left. The region where the two nuclei fuse, and where the two crescents are thickest, is in the eventual posterior and ventral part of the embryo, while the opposite side of the egg is the anterior-dorsal.

Shortly after fertilisation, the clear cytoplasm spreads over the greater part of the animal half of the egg. The original central material of the unfertilised egg, the grey yolky plasm, is now confined to the vegetative region. It gradually becomes differentiated into a darker mass at the vegetative pole, and a lighter grey material which forms a crescent at the opposite side of the egg to the yellowish stuff. The egg thus comes to have a fairly complicated architecture.

These various regions of the egg are highly significant for the future development. By careful observation, Conklin could follow each region through the subsequent stages, and determine which organs it eventually formed. The end-product which a given region of the egg (or early embryo) will eventually form if left to itself in the intact egg is spoken of as its 'prospective fate' (sometimes the expression 'presumptive fate', derived from the German, is used). Conklin was thus in a position to make a map of the early cleavage stages, marking on it the prospective fate of the various parts; in fact, in *Styela*, the map was more or less made for him by the colouration of the various regions. This is not usually the case. In *Amphioxus*, for example, although the general set-up is probably

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released a clear grey cytoplasm. The yellow cytoplasm is accumulating at the vegetative pole.

(*c*) The sperm nucleus *s* is visible in the yellow cytoplasm which lies at the vegetative pole. The clear grey cytoplasm has moved down and lies just above the yellow.

(*d*) Shortly before the first cleavage. The sperm nucleus has moved up and met the female nucleus just below the equator. The yellow and grey cytoplasms have moved up with the sperm nucleus. The yellow crescent is more or less superficial, the grey one extends into the depth of the egg.

(*e*) Two-cell stage. The grey crescent is becoming more diffuse.

(*f*) Eight-cell stage from side.

(*g*) Sixteen-cell stage from animal pole. Note the bilateral symmetry.

(*h*) Early gastrula from vegetative pole, looking into the wide open blastopore *b*; on the dorsal side of this are the future neural cells *n.p* and chordal cells *c*.

(*i*) Longitudinal section through young larva. *n.p.* neural plate, *c* notochord, *e* ectoderm, *end* endoderm lining the archenteron, *m* mesoderm, *b* blastopore. (In this and Figures *f*, *g* and *h* the yellow crescent material is shaded so that its movements can be followed but it cannot actually be recognised by its colour so clearly as the diagram suggests.)

(*j*) Longitudinal section through a young larva: *c.*, notochord; *ey.*, eye spot; *end.*, endoderm; *t.e.*, endoderm of tail; *mes.*, mesenchyme; *n.*, nerve cord; *n.v.* neural vesicle; *pap.*, adhesive papilla.

(After Conklin 1905.)

almost identical with that in *Styela*, only the peripheral cytoplasm can be distinguished (although it corresponds with the yellowish material in *Styela* it is grey in *Amphioxus*). In most other organisms there is no overt sign which distinguishes the regions of different prospective fate, and much ingenuity has had to be used to keep track of the parts of the early egg and discover where they get to and what they develop into. In spite of the technical difficulty of obtaining them, prospective fate maps are the clearest way of summarising the future course of development, and we shall use them again and again to show how the embryological events

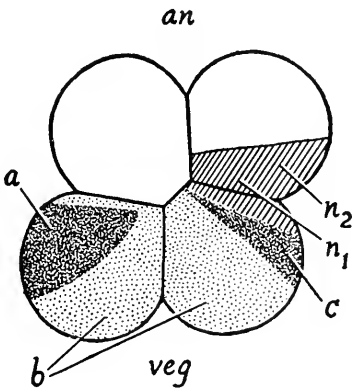

FIGURE 7.2

Presumptive areas in the 8-cell stage of an ascidian, seen from the right side. White, ectoderm; *a*, mesoderm (muscle); *b*, endoderm; *c*, notochord; *n*, spinal cord; n_2, brain. (After Vandebroek 1938, Reverberi 1948 and Ortolani.)

which look so different in different groups of vertebrates, can really be traced back to the same general scheme.

The prospective fate map of the ascidians, and of *Amphioxus* which is essentially similar, is shown diagrammatically in Fig. 7.2. It has recently been carefully studied by Vanderbroek (see Reverberi 1948) and Ortolani (1954). It will be seen that the yellow crescent of *Styela*, which corresponds to the grey crescent which alone is distinguishable in *Amphioxus*, eventually becomes mesoderm. It is thus not the constituent which forms the main body axis, since that is composed of the neural plate with its underlying chorda. The original locations of these two organs are found on the other side of the egg, in the light grey area of *Styela*, which does not appear distinctly till after fertilisation. We shall see (p. 146) that in Amphibia it is

the material for the chorda, more or less corresponding to this light-grey crescent, which first becomes visibly distinguishable; it is important to remember this difference between the two forms, which we shall find are otherwise very similar in their general pattern. Above the ring formed by the mesodermal and chorda-neural crescents lies an area which will form ectoderm, and which in *Styela* can be seen to originate in the clear cytoplasm which emerged from the ruptured germinal vesicle; below lies the dark-grey yolky material which develops into endoderm.

It is now necessary to trace the movements and changes by which the prospective areas attain their fate. These are perhaps slightly clearer in *Amphioxus* (Fig. 7.3), but essentially the same features can also be seen in the ascidians. The first cleavage plane, as has been said, lies in the plane of bilateral symmetry which bisects the mesodermal and chorda-neural crescents, and runs through the animal and vegetative poles. The second plane is also vertical and is at right angles to the first, cutting off two slightly smaller cells at the posterior side, and two larger ones in front. (It was in regard to the orientation of this and the later stages that the earlier workers were mistaken.) The next cleavage is horizontal, giving an 8-cell stage in which the lower group of cells are all slightly larger than the corresponding upper ones. Further than this it is unnecessary to follow the cleavages in detail. As they proceed, a jelly-like material accumulates in the centre of the mass of cells, which are gradually pushed outwards to form a hollow sphere, which is a blastula of the typical form we have already seen in the echinoderms.

The gastrulation process, by which this blastula becomes converted into a three-layered embryo, begins by a slight flattening of the vegetative end. The cells in this region are those derived from the dark-grey yolky material and are somewhat larger than any others in the blastula, so that they form a rather solid-looking coherent flat plate. This sinks into the interior of the hollow blastula. The opening which leads in from the exterior towards the sunken plate is the blastopore, its edges the blastoporal lips. The cavity into which it leads, which grows deeper as the plate sinks further into the interior, is the primitive gut.

The shape of the blastopore alters somewhat as the gastrulation proceeds, but there is no need to repeat here all the details given by Conklin in his classical account of *Amphioxus*. At first the endoderm plate is triangular, with a wide straight dorsal lip and two lateral boundaries which converge towards the ventral lip. Although the sinking in of the endoderm begins ventrally, it proceeds fastest on the dorsal side, and it is the dorsal lip which is the most sharply inflected. In *Styela* this lip is originally made up of about six transverse rows of cells, each row containing rather more

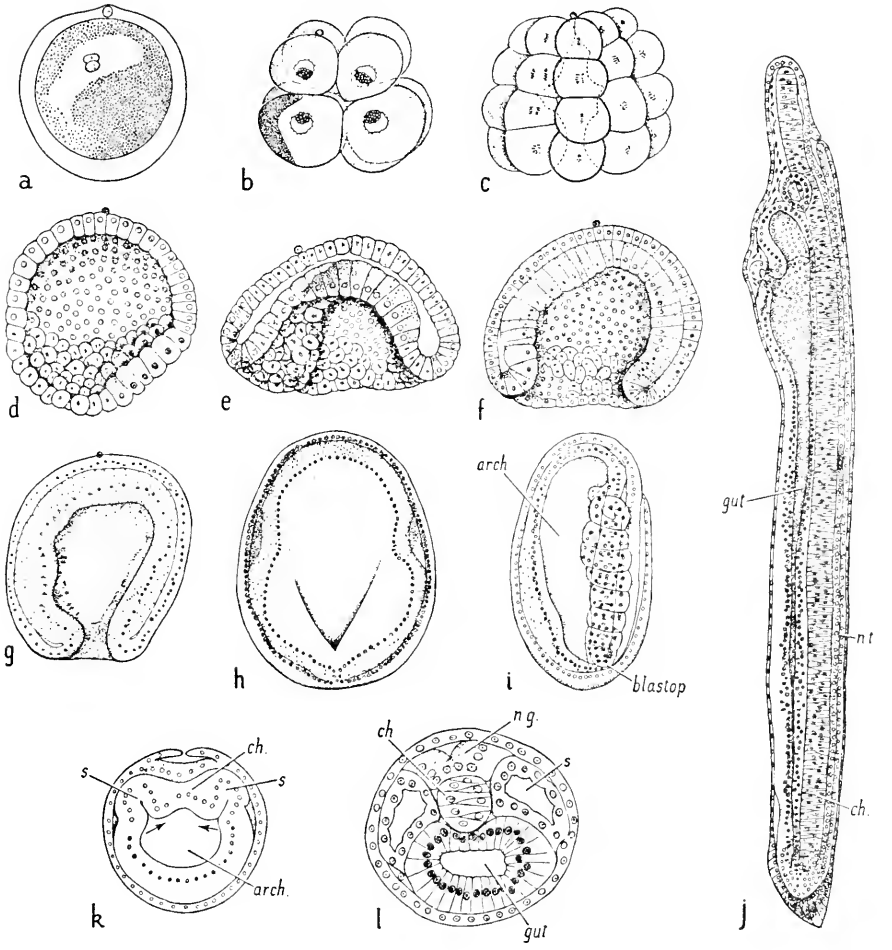

FIGURE 7.3

Development of *Amphioxus*.

(*a*) Section through egg one hour after fertilisation, showing the conjugation of the pronuclei, a polar body, and the grey crescent material on the left (posterior) side.

(*b*) Eight-cell stage, seen from right side.

(*c*) Thirty-two-cell stage. A space is appearing in the middle of the group of cells.

(*d*) A left half of an early gastrula, seen from the right side. Note large blastocoel cavity, the plate of large endoderm cells on the lower right (anterior) side of the drawing and the irregular, rapidly dividing cells of the mesodermal (=former grey) crescent on the lower left.

(*e*), (*f*) Similar views of later gastrula stages.

(*g*) Gastrulation nearly completed and the blastopore narrowed to quite a small hole.

than a dozen cells, all of which have been derived from the light-grey chorda-neural crescent. The lower three rows lie inside the lip, and come into the roof of the primitive gut; these give rise to the chorda, while the remaining three rows stay on the outer surface and become the neural plate. During the gastrulation, the arrangement of the cells in the rows is profoundly altered; they slip between one another, so that the chorda and neural plate, whose material was originally arranged in transverse rows, become longitudinal strands running forward from the blastopore lip. The effect of this is that the cells of the mesodermal crescent on the other side of the egg have to move over towards the dorsal side to fill the gap which would otherwise be left; and as the stretching of the dorsal organs continues, the primitive gut becomes a tube with a strip of chorda along its most dorsal part, a strip of mesoderm on each side of that, and the main hollow of the tube lined with endoderm.

As the elongation proceeds, the blastopore narrows like the mouth of a laundry bag when the string is pulled tight. Before it is completely closed in *Amphioxus* the ectoderm just above the ventral lip grows up in a curved ridge, the sides of which rapidly come together, covering the still-open blastopore from sight, and progress forwards above the neural plate, like two flaps being drawn together by a zip-fastener up the mid-dorsal line. Underneath these flaps, the neural plate continues to get longer and narrower, and its centre sinks down to form a trough or groove; in the most anterior end, indeed, it rolls up completely so as to produce a neural tube, such as we shall find in the higher vertebrates. Meanwhile important changes are beginning in the walls of the archenteron. These can be most simply described by saying that the endoderm, which in a cross-

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(*h*) A dorsal view just after the completion of gastrulation. The blastopore is at the bottom, but is covered by the two flaps of ectoderm which are growing up over the neural plate, leaving a pear-shaped area of it visible. The small circles arranged in a figure of eight are the nuclei in the endoderm cells lining the archenteron.

(*i*) Optical section at a slightly later stage, in the same orientation as *g* and the previous drawings. Dorsal to the archenteron (*arch.*) the left row of somites is seen lying above the notochord, and dorsal to that is the neural plate, covered by the flap of ectoderm; *blastop*=blastopore.

(*j*) Left view of 48-hour larva: *gt.* gut; *n.t.*, neural system; *ch*, notochord.

(*k*) Section through the level of the second somite in Figure *i*, showing the neural plate (dotted) beneath which are the notochord, and the somites which are folding off from the walls of the archenteron.

(*l*) Section through same somites somewhat later. The neural plate is folding into a groove; the somites have separated from the gut-wall and from the notochord, and the coelomic cavity has expanded within them.

(After Conklin 1932.)

section still makes up only the ventral and lateral walls of the gut tube, now starts to grow inwards from each side towards the dorsal midline. In doing so, it first undercuts the mesodermal strips, pushing them outwards as grooves which eventually become completely cut off from the gut as hollow sacks; and after pushing off the mesoderm, it then grows under the chorda, excluding it also from the walls of the gut, which thus becomes completely lined by endoderm. It should be noted that although this is a convenient method of describing what happens, we do not actually know that it is primarily the endoderm which pushes off the mesoderm, and not the latter which actively withdraws itself; such questions could only be definitely answered by an experimental analysis which has not yet been made. It may well be the mesoderm which plays the active part in its removal from the gut wall, since soon after this it undergoes a change for which the endoderm can scarcely be made responsible; the long hollow sacks of mesoderm become nipped off into a series of short, square chambers, which lie in orderly pairs on each side of the chorda; these are the somites.

Only the first eight or nine pairs of somites are formed in this way as outpushings from the gut wall. By the time they are laid down, the blastopore, hidden under the ectodermal fold, is nearly closed. The further growth in length of the larva is brought about by the proliferation of all the material lying round this remnant of blastopore. This mass of rapidly dividing cells grows out posteriorly, differentiating directly as it does so into neural plate, notochord, somites and endodermal gut wall. It has been suggested that in higher vertebrates too the more posterior regions of the body are formed from a general proliferating mass in which the embryological processes are by no means so clear-cut as they are in more anterior regions; but we shall see (p. 263) that recent work makes this unlikely.

There is no need for our present purposes to follow the later history of the *Amphioxus* larva in detail. It should perhaps be mentioned that the mouth forms as an opening which breaks through into the gut near its anterior end, while the anus is a similar opening nearer to the site of the blastopore remnant, but somewhat anterior to it. The somites, from a fairly early stage, expand laterally, growing round between the ectoderm and endoderm till they fuse in the mid-ventral line, and thus provide the gut with a complete mesodermal clothing. These lateral extensions of the somites are, from the first, hollow like the somites from which they come, and the space between their inner and outer walls is the origin of the final body-cavity or coelom. If this cavity is traced back, it will be found to be derived directly from the cavity of the gut or enteron; whence a

body-cavity originating in this way has been called an entero-coel, in distinction to the schizo-coel (formed by splitting of an originally solid layer) which is characteristic of most vertebrates. But it seems probable that the distinction is not such a fundamental one as some of the older embryologists thought.

The eggs of ascidians have been favourite material for experimental study for many years; *Amphioxus* material is less easily come by and much less is known about it, though what data do exist suggest that its epigenetic physiology is not greatly different from that of the better-known group. The older workers found much evidence that the ascidian egg is a typical mosaic type. In particular, Conklin (1931) showed that after fairly strong centrifugation which moved around the interior cytoplasm abnormal larvae develop, their build corresponding to the new positions to which the ooplasms had been shifted. He found, however, that the essential factors which determine the mode of development of the regions are not the inclusions to which the colours of the various crescents are due. For instance, the yellow granules can be moved out of the yellow crescent by mild centrifugation, but the mesoderm still appears in its normal place so long as the hyaline ground-substance has remained in place. Ries (1939, see also Reverberi and Pitotti 1939) later found that the property of developing into mesoderm (and particularly muscles) is bound up with some cytoplasmic constituent which has a high peroxydase activity; possibly these are ultra-microscopical granules. Wherever the peroxydase-containing material is forced to by the centrifugal force, there the muscles will eventually develop.

In recent years, it has become clear that the location of specific ooplasms in definite regions of the egg is only one part of the story (Review: Reverberi 1948). The first important additional information concerned the mechanism by which the localisation is brought about. Dalcq (1932) found that, if eggs are cut into two parts before being fertilised, two complete larvae can be produced (provided the cut is meridional, so that each fragment contains the whole animal-vegetative axis). This is a typical 'regulation', similar to that found in the echinoderms. It certainly indicates that considerable redistribution of the ooplasms can occur in the unfertilised egg. Reverberi (1936) in similar experiments studied the capacity of egg fragments to regulate sufficiently to carry out a normal bilaterally symmetrical cleavage. He found that they retained this degree of flexibility up to the time of the emission of the polar bodies, but that the regulation is dependent on some factor located in the vegetative region, in the absence of which the animal part alone cleaves as though it remained only part of an egg, and does not develop bilateral symmetry. Thus in

the egg before and just after fertilisation there is a considerable capacity for regulation, which can be shown to involve mutual interactions between one part and another.

Evidence has also accumulated that even after the ooplasms have become localised, reactions between the parts of the embryo are by no means at an end. The details of Dalcq's experiments with fragments of unfertilised eggs had already suggested this possibility to him, and conclusive evidence of regulation in the 2-cell stage was found by von Ubisch (1938), who was able to cause two eggs at this stage to fuse together, when in some cases only a single perfectly normal larva was developed.

Much fuller information has been obtained from experiments in which, at the 8-cell stage, the blastomeres have been separated and recombined in various ways (cf. Rose 1939, Reverberi 1948, Reverberi and Minganti 1953). Quite a complicated cross-fire of interactions has been discovered. If the first eight cells are separated into couples, as in Fig. 7. 4a, development is not fully mosaic, since no brain, adhesive organs or eye-spots appear in the larva from the anterior animal couple, nor any spinal cord from the anterior vegetative couple. The other experiments summarised in that Figure show that the brain, eyes and adhesive organs are induced to develop from the anterior animal cells by some influence proceeding from the anterior vegetative couple, but this influence is not effective on posterior animal cells. In the standard embryological terminology, we should say that only the anterior animal cells are *competent* to react to the stimulus from the anterior vegetative blastomeres. There is some evidence that the interaction is still more complex, in that there may be an influence from the posterior vegetative blastomeres tending to inhibit the development of the brain in the cells immediately above, even if these are an anterior couple. The formation of the spinal cord of the nervous system is also dependent on reactions between different regions, but in this case it is a derivative of the anterior vegetative blastomeres which does not develop when isolated from the anterior animal cells.

We therefore find that, far from the ascidian egg being a complete mosaic, it first undergoes a period in which a number of ooplasms become localised, and then enters one in which, although several of the regions are already endowed with a capacity for independent differentiation, some others are still labile, and develop normally only if certain reactions take place. This lability affects particularly the nervous system, which has two rather distinct parts, the brain and spinal cord, while the eye-spots and adhesive organs also become fixed in their developmental fate at the same time. We shall see that in vertebrate embryos the development of the nervous system, and of many other organs which depend on it, is

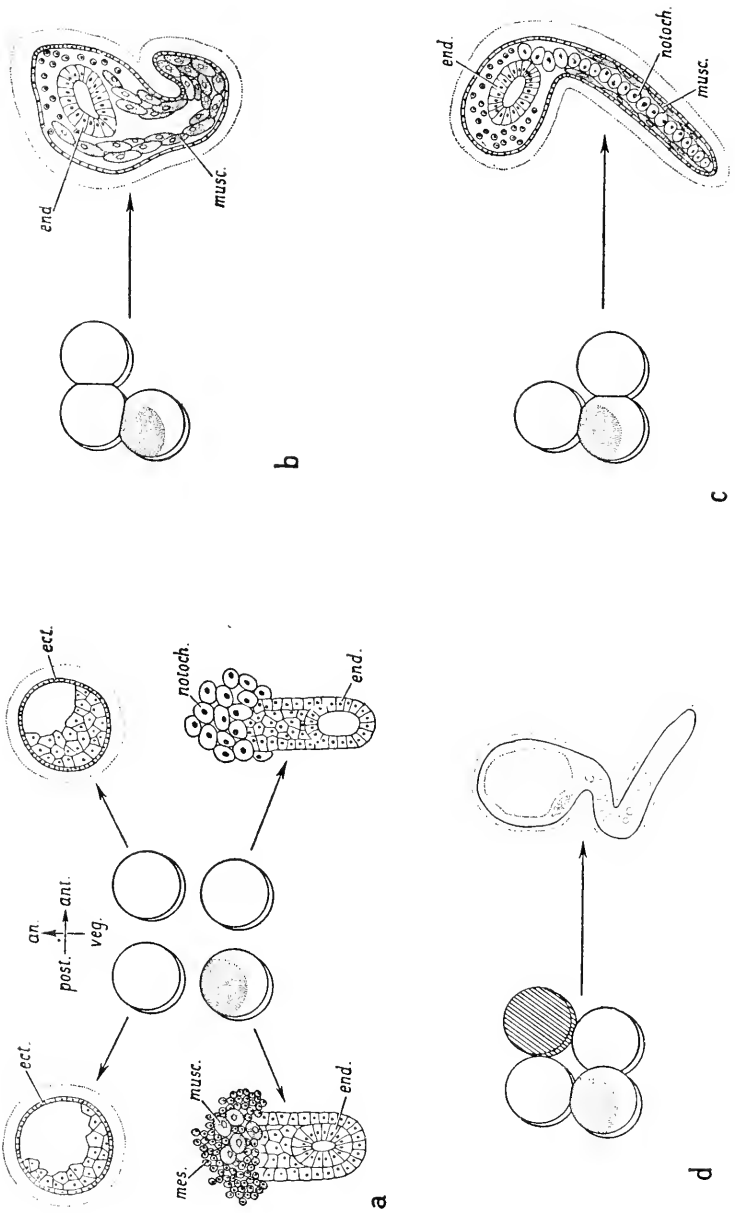

FIGURE 7.4

Isolation and grafting of blastomeres in ascidians.

(*a*) Separation of the 8-cell stage of an ascidian into four pairs of cells. The two posterior animal cells give ectoderm, and the two posterior vegetative ones mesoderm, including muscle, and endoderm; this corresponds with their prospective fate. But the anterior animal ones give only ectoderm, with

the result of a process of induction, and we can see the first signs of this in the ascidians. But in the ascidians the primary inductions occur during the early cleavage divisions, while in the higher vertebrates they happen much later.

SUGGESTED READING

The papers of Conklin 1905, 1932, are classics of descriptive embryology. In addition Rose 1939, Reverberi 1948, Dalcq 1938, pp. 103–27.

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no brain or palps, and the vegetative anteriors endoderm and notochord but no neural tube.
(*b*) If the anterior vegetative cells are removed, the rest does not develop the brain and palps (although the anterior animal cells from which they should form are still present), nor neural tube.
(*c*) If the anterior animal cells are removed, and the brain and palps are again absent.
(*d*) The anterior animal couple are removed and replaced by a posterior animal couple (shaded). Still the brain and palps are absent.
(After Reverberi 1948.)

CHAPTER VIII

THE INSECTS

THE GREAT group of insects contains an enormous range of different forms, and any treatment of insect development which can fit into the framework of this book must be an extremely summary and simplified one. There is not only a very wide range of different types of embryonic development to be covered, but our knowledge of the phenomena of insect metamorphosis is considerable, and, although we have neglected this problem in the annelids and echinoderms, it seems desirable to give some account of it in insects. As is well known, there are all gradations in the intensity of the changes involved in metamorphosis. In some primitive insects (the Ametabola), the larval form develops gradually into the adult, with no sudden or marked change. There are other more complex types, in which the wing buds may be exposed in the larval stages (Exopterygota) or concealed beneath the surface until the time of metamorphosis (Endopterygota); and again the larva (in this case often known as a 'nymph') may be directly transformed into the adult (Hemimetabola) or there may be a pupal stage intercalated (Holometabola). In the extreme type, the Holometabola, the adult organism may have almost no immediately obvious similarity to the larval; the life-history comprises two very distinct developmental systems. Since there will be no space to treat all the intermediate conditions, we shall find it convenient to deal first with the embryonic development, by which the larva is produced, and then to pass on to the processes of metamorphosis, particularly in those types in which they are most intense and far-reaching.

Embryonic development

There are many types of insect eggs (Review: Johannsen and Butt 1941), but they are all variations on a fundamental plan which is at first sight rather unlike that of any of the other eggs described in this book (although related to those of other arthropods, which we shall not consider). Their most obvious characteristic is that the yolk, instead of being concentrated at the vegetative end of the egg, is more or less uniformly distributed throughout the whole central region of it; whence the eggs are often referred to as 'centrolecithal' in contrast to the 'telolecithal' eggs of other groups. Around the central mass of yolk, there is always a cortex, or peripheral sheet of cytoplasm, which may be quite thin, but is sometimes

fairly thick. In recent years, the importance of the cortex in other eggs, such as those of the echinoderms, the spiral-cleaving forms, and the Amphibia, has come to be recognised, and with this recognition, the conditions in the insect egg begin to seem less peculiar than they had done previously. Instead of their organisation being fundamentally different from that of other groups, we see that they differ only quantitatively, in that their cortical plasma is rather thicker than usual, and their yolk more evenly distributed within the interior cytoplasm.

Insect eggs often exhibit an obvious bilateral symmetry, the dorso-ventral plane being built into them during their formation in the ovary. They also tend to be provided with rather rigid external membranes, through which a micropyle leads the sperm in to the egg. The vitelline membrane, formed by the egg itself, is often partly chitinised and highly impermeable to most solvents, a circumstance which makes fixation difficult and renders the eggs troublesome objects for detailed study.

Although as we have seen the structure of the insect egg is not entirely dissimilar to that of other groups, it is nevertheless peculiar enough to cause profound modifications in the cleavage. Insect eggs, in fact, do not cleave at all in the normal sense of the word. After fertilisation the gamete-nuclei conjugate somewhere in the neighbourhood of the micropyle, and the zygote nucleus which is thus constituted divides a considerable number of times without any accompanying division of the main mass of egg cytoplasm. Each nucleus, however, is surrounded by a small region of relatively non-yolky cytoplasm and at each nuclear division, this divides into two. The nuclei, accompanied by their patches of cytoplasm, move away from one another as though repelling each other, and usually go through a stage in which they are arranged roughly as a hollow sphere, or at least a hollow shape more or less conformable to the outline of the whole egg (Fig. 8.1).

After some time, most of the nuclei reach the cortex and pass into it, their cytoplasmic halos fusing with it. It is not until the cortex becomes populated with nuclei that cell boundaries make their appearance, and they form only in the cortex itself, which thus becomes converted into an epithelium enclosing the main mass of yolk (which still contains a few nuclei, whose later function is to take part in the digestion of the nutritive materials). The enclosing epithelium is known as the *blastoderm*, and it is from this that the embryo develops.

The first sign of the embryo is a thickening of the blastoderm. This may at first be double, so that there are two thicker strands, with many nuclei, rather like the embryonic bands which form in an annelid which develops without any larval stage, such as *Tubifex* (p. 96). Soon, however, the

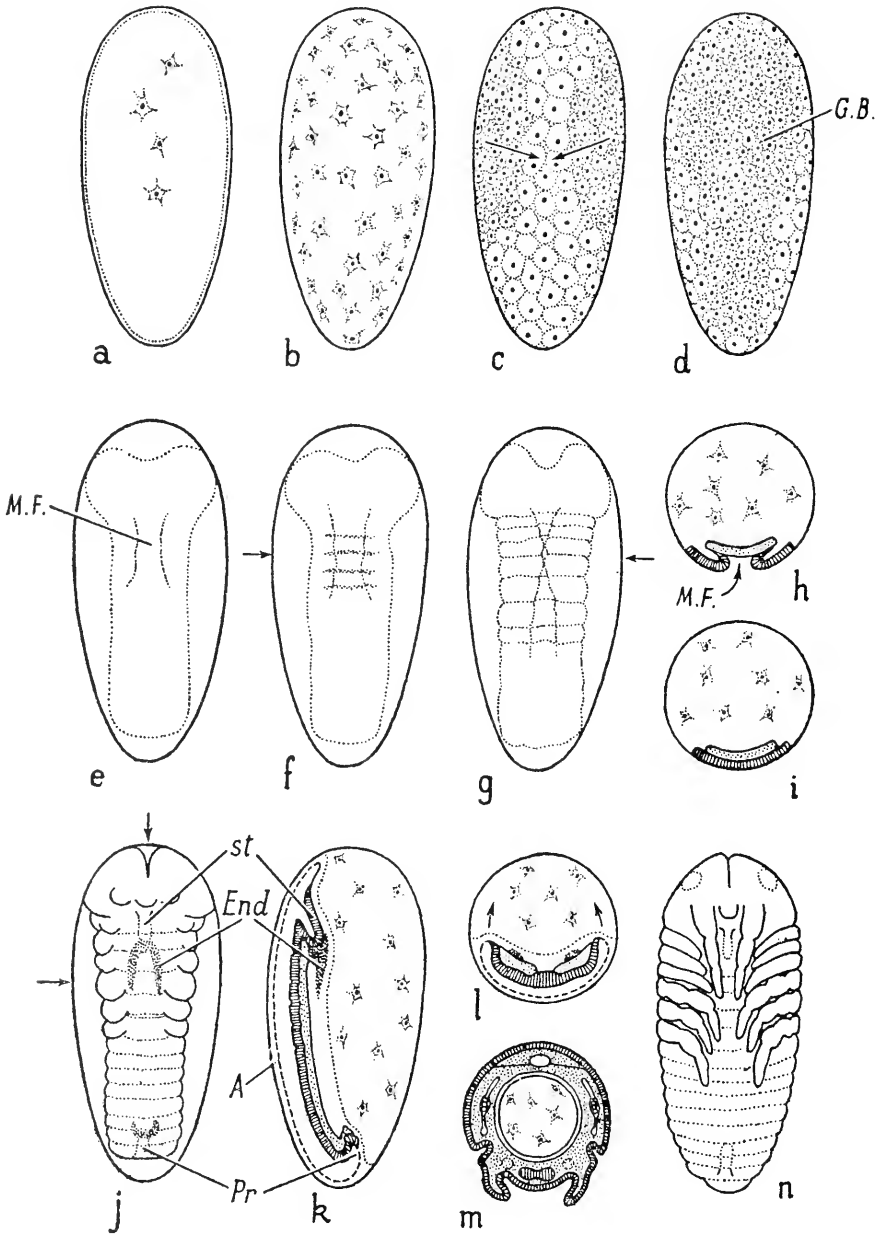

FIGURE 8.1

Generalised diagram of insect embryonic development: (*a*) is a section showing the cleavage nuclei, which collect at the surface to produce a blastoderm (*b*). The number of cells increases, and two densely aggregated groups appear (*c*). These come together and fuse to form a single germ-band (*d*). Figures

bands come together and form a single elongated thickening, the so-called 'germ-band'. The midline of the germ-band is the mid-ventral line of the final animal; in insects, it is the ventral side, not the dorsal, which plays the dominant role.

Along this mid-ventral line, the germ-band folds downwards into a groove whose edges come together to constitute the ectoderm, while the floor of the groove disappears below the surface, as a lower layer or 'hypoblast', which eventually gives rise both to mesoderm and to the endoderm of the mid-gut. At the anterior and posterior ends of the germ-band, pockets are pushed in along the length of the embryonic body, forming respectively the mouth and anus, or, more correctly, the stomadeum and proctodeum. From these, more endoderm is produced, to develop into the foregut and hindgut.

There is one other important group of internal cells which may be mentioned. In many insects, (particularly in the 'determinate' type to be mentioned below), the cells at the most posterior end of the blastoderm have a special character, distinguishing them histologically from the rest. They are known as the 'pole cells', and they later migrate into the gonads and become the source of all the later-formed gametes. If they are removed, by cauterising or otherwise killing them at an early stage, completely sterile individuals may be produced, and it seems that in some forms at least they are the only cells of the embryo which are capable of differentiating into gametes. The whole developmental sequence of mother- and daughter-cells, from the pole cells to the final gametes, is sometimes known as the 'germ-line', a term to be carefully distinguished from the 'germ-band' which, as we have seen, is used for the blastodermic thickening from which the embryo arises.

While the lower layers of the embryo are forming in the way described

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(*e*), (*f*), (*g*) show the outline of the germ-band, with the longitudinal furrow in which the mesoderm is invaginated (*M.F.*) and the transverse furrows delimiting the segments: the arrow at the side marks the first intersegmental furrow, which is between the most posterior head-segment (second maxillary) and the most anterior thoracic; (*h*) and (*i*) show sections through the stages of (*e*) and (*g*) at the level of the arrow to show the invaginating mesoderm (dotted) becoming covered by ectoderm (lined). The next stage is shown in surface view (*j*) and longitudinal section (*k*); the stomodeum (foregut) and proctodeum (hindgut) are pushed inwards from the surface, and from the former endoderm arises and will develop into the midgut; the appendages are beginning to appear on the segments of the embryo. Figures (*l*) and (*m*) are transverse sections of rather later stages to show how the edges of the embryonic area grow round to enclose the yolk and thus provide the dorsal surface of the completed embryo, which is drawn in (*n*)
(After Seidel 1936.)

above, the germ-band is becoming transversely segmented, and from each of the anterior segments, outgrowths protrude and grow into the cephalic and thoracic appendages of the larva. All the main organs of the embryo are then at least indicated, though they still have much histological differentiation to carry out before they are fully developed. By the time the main blocking-out of the embryo is complete, the germ-band may still represent only the ventral surface, the dorsal side being occupied by the mass of yolk. At some stage, often quite a late one, the body wall is completed by the outward growth of the two lateral edges of the germ-band, which eventually meet and fuse at the mid-dorsal line. This finishes the laying down of the embryo proper.

In some insects, more or less elaborate extra-embryonic structures are also developed. There are often membranes which cover the embryo and presumably help to protect it from outside influences. These are derived from parts of the blastoderm peripheral to the germ-band, by the formation of folds which eventually meet and fuse above the embryo, leaving an outer layer (or serosa) and an inner (or amnion). There is another and most peculiar phenomenon to be mentioned, which however plays no part in the actual formation of the embryonic organs, namely the so-called 'blastokinesis'. This is the name for a series of movements by which the germ-band may at some stage be dragged from the surface right into the middle of the yolk mass, only to emerge on to the surface again later. The most strongly developed blastokinetic movements occur in species in which the embryo is small compared with the size of the whole egg, but even in forms in which the embryo occupies almost the entire available space, there are often considerable shiftings of the blastoderm as a whole (cf. Fig. 8.2).

As was pointed out above, there is within the insect kingdom a very large range of variations around the generalised type which has just been described. These variations fall into a series, from an 'indeterminate type' at one end to a 'determinate type' at the other (Seidel 1936). The indeterminate type includes the eggs of some of the more primitive groups of insects, such as Orthoptera and Odonata; it is characterised by eggs which are usually rather large and often elongated, provided with only a thin cortex, and developing an embryo which is small in relation to the egg. The name 'indeterminate' is used because very considerable regulation is possible during the early stages of development. In the determinate type, regulation is very slight or altogether absent. The most typical representatives of this group are the Lepidoptera and Diptera, whose eggs have a thicker cortex, which is often regionally specialised even before fertilisation. The embryo normally occupies the whole, or at least the greater part

of the space available to it. Between these two extremes there are various intermediate types of eggs, of which those of the Hymenoptera and Coleoptera are the best known.

An experimental study of development in the related group of Arachnids has recently been published (Holm 1952).

2. *Experimental analysis of some types of insect development*[1]

It is the indeterminate type, with its capacities for regulation, which allows us most easily to gain an insight into the epigenetic system of the insects. We shall therefore begin by considering a typical representative of this type, the dragon-fly (Odonata) *Platycnemis pennipes* (Seidel 1929, 1936).

The cleavage of the nuclei, their repulsion from one another, and the eventual formation of a blastoderm covering the whole surface proceed in an absolutely typical manner (Fig. 8.2). A germ-band then appears, in the form of a region of the blastoderm which is thicker and contains a higher concentration of nuclei. It at first shows some signs of doubleness, but soon shortens somewhat and becomes a single area except in its most anterior part, where there are two lobes which will eventually develop into the head. The next stages of differentiation—that is, the further thickening of the germ-band, the folding inwards of the lower layer, the formation of transverse segments and the appearance of the appendages—all begin in a region which lies a short distance posterior to the head, in an area which later becomes the anterior thorax, and they spread both anteriorly and posteriorly from there until they affect the whole germ. This region, where development is visibly most advanced, is known as the Differentiation Centre; we shall see that it has important physiological functions as well as being morphologically in the lead. During the development of the embryo, a considerable blastokinesis occurs, the whole germ being at first folded deep into the yolk, and then emerging again, at the same time twisting around its longitudinal axis, so that it eventually lies with its dorsal side against the same part of the egg membrane as was originally in contact with its ventral face. The dorsal wall is not actually completed until after these blastokinetic movements have ceased.

The early stages of the embryo have very considerable powers of regulation, and two complete embryos can sometimes be produced by a single egg if, in some way or another, the developing system can be broken into two effectively separate parts. A mechanical bending of the egg is in some cases sufficient to split the yolk into two parts, and if this happens

[1] General reviews: Seidel 1952*a*, 1953; Richards and Miller 1937, Krause 1939.

at an early stage of the dispersion of the nuclei, each part forms its own complete embryo. Owing to the extensive movements which take place during development, the relations of these twins to one another may become very peculiar. In one case described by Seidel, a slightly oblique fold had separated the egg into a larger and a smaller portion. Each

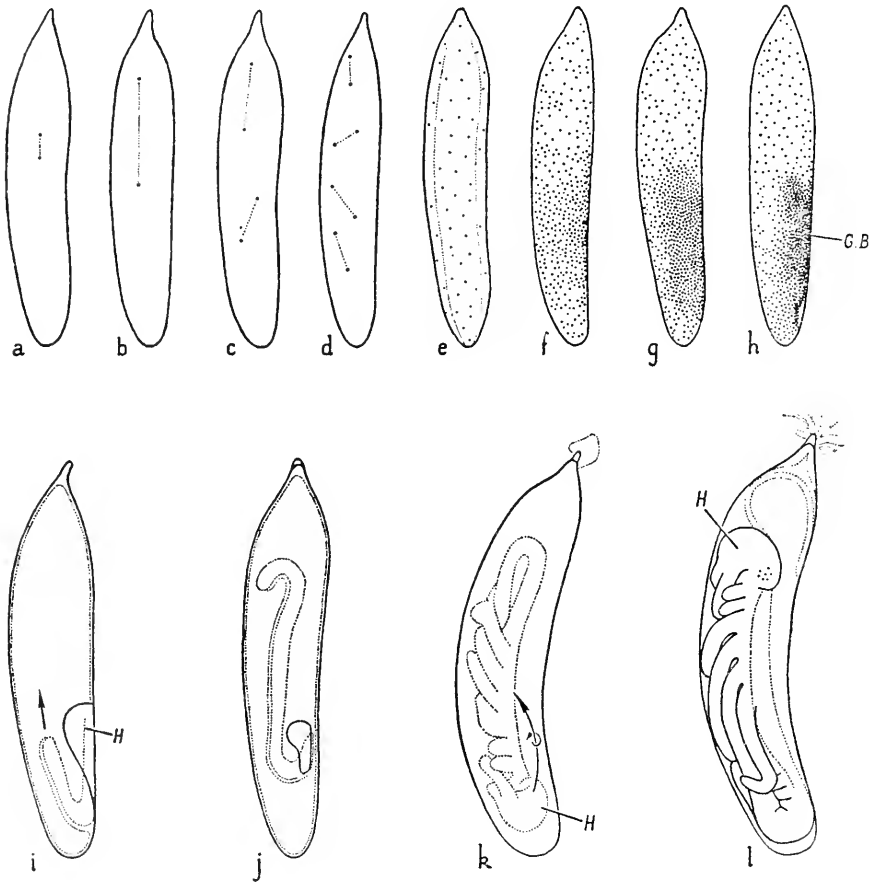


FIGURE 8.2

Development of the dragonfly *Platycnemis*. Figures *a*, *b*, *c*, and *d* are sections showing the first three divisions of the nucleus, and the spreading of the daughter nuclei through the egg cytoplasm (the two sister nuclei from a division are joined by a dotted line). In *e* the nuclei are beginning to reach the surface; *f*, *g* and *h* are surface-views showing the multiplication of the nuclei to form a blastoderm and their aggregation to produce the germ-band (*G.B.*). In *i* the germ-band is being pulled into the interior of the egg; at the stage of Figure *j* nearly the whole embryo (dotted) lies internally. It is then pulled out again, at the same time rotating around its longitudinal axis (*k*). Note that in its final position (*l*) its head (*H*) is again towards the pointed end of the egg. (After Seidel 1929.)

developed an embryo, and the two twins must at first have lain back to back. As each spread out from the original ventral rudiment to close in and cover their dorsal sides, the larger engulfed the smaller and, moreover forced it to roll up the wrong side out. Thus the egg finished by containing a large embryo which enclosed a smaller twin which was inside-out, surely one of the most remarkable arrangements ever produced (Fig. 8.3).

More insight into the causal processes of development is gained from experiments in which the egg is divided in two transversely. This can be done either by killing off one end by burning with a microcautery, or better by constricting the egg with a loop of hair so that it is divided into two parts. By using these methods, Seidel showed that during the dispersal of the cleavage nuclei there is a small region at the posterior end which is essential for all further development, since if more than a very

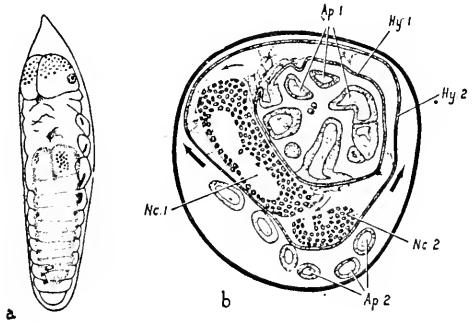


FIGURE 8.3

An internal twin in *Platycnemis*, following a longitudinal split in the egg during the cleavage stages. Both longitudinal halves produced an embryo, and as the larger germ-band grew round dorsally to enclose the yolk (direction of thick arrows) it reversed the dorsal closure of the smaller one (which should have grown round in the direction of the thin arrows). In (*a*) the internal embryo is shown dotted. In the transverse section (*b*) note that the internal embryo is inside out, with the appendages (*Ap.* 1) inside and the nerve-cord (*Nc.* 1) outside the hypodermis (*Hy.* 1); the organs of the external embryo are at *Ap.* 2, *Nc.* 2 and *Hy.* 2. (After Seidel 1936.)

small fraction of this end is completely removed, the egg forms a blastoderm but never proceeds to develop a germ-band or embryo. This essential posterior region was named the 'formation centre' (Bildungszentrum' in German). Its activity is almost certainly concerned with the production of a diffusible chemical, since if the egg is constricted by a hair which is not pulled completely tight but leaves a small channel through

which diffusion could take place, the formation centre can still be effective and the anterior part develop. This only occurs, however, if the constriction is made after the formation centre has become populated with the dispersing cleavage nuclei. If, at an earlier stage, one makes a partial constriction which is loose enough to permit diffusion of chemical substances but too tight to allow nuclei to pass, the formation centre can never become nucleated, and it appears to be quite ineffective, since no embryo is developed. One must conclude that the formation centre consists of some specialised cytoplasm at the posterior end, but that it requires to be activated by the passage into it of a cleavage nucleus (together with the accompanying cytoplasmic halo); after being activated, the formation centre gives out a chemical substance which diffuses forwards and enables the main part of the egg to develop (Fig. 8.4).

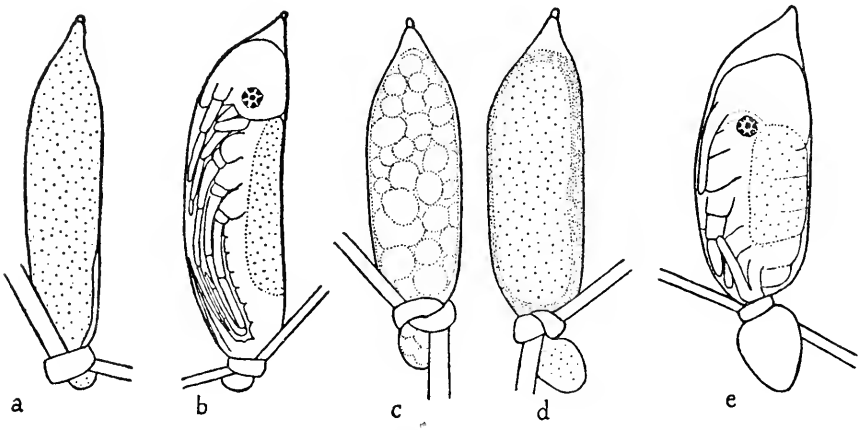


FIGURE 8.4

The operation of the Formation Centre. If a very small part of the posterior of the egg is constricted off at an early stage (*a*), an embryo can develop (*b*); but if the constriction lies a little further forward (*c*) no embryo forms (*d*). After the formation of the blastoderm, however, an embryo is formed even if the constriction lies well forward (*e*), since the Formation Centre has by that time completed its action. (After Seidel 1929.)

Seidel was able to alter the regular process of dispersal of the cleavage nuclei, either by killing one with localised ultra-violet irradiation or by partially ligaturing the egg for some time so that the dispersal had to occur in an abnormal space. He could in this way cause the formation centre to be invaded by a nucleus other than that which would normally have reached there; and he showed that any nucleus—or at least any of

the 128 which are formed in the first seven divisions—is adequate to activate the centre.

The chemical which diffuses forwards from the formation centre does not act directly on each separate part of the egg. It sets going the differentiation centre, which is not only morphologically distinguishable, as the site of the first steps in the development of the germ-band, but is also the second centre of physiological activity. Once it is activated by the substance from the formation centre, it becomes a focus around which the embryo is organised. The region over which its activity extends can also be broken by constricting the egg with a hair loop, but in this case the continuity is much more easily disrupted, and even a fairly loose constriction is enough to inhibit the passage of the differentiation centre's influence. Thus if a loose loop is tied round the egg posterior to the differentiation centre, between it and the formation centre, an embryo develops only in the anterior region; if the loop lies anterior to the centre, the embryo lies wholly posterior to the loop, while if the constriction is located actually within the differentiation centre, twin embryos will form, one in each part (Fig. 8.5).

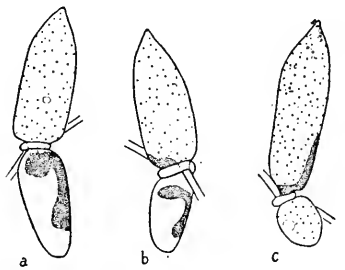


FIGURE 8.5

The action of the Differentiation Centre in *Platycnemis*. If a loose constriction is made in front of the Centre (*a*) the embryo forms wholly posterior to it. If the constriction is behind the Centre, the embryo lies wholly in front of it (*c*). If the constriction is at the actual site of the Centre, this may be divided and embryo formation take place both in front and behind (*b*). (After Seidel 1938.)

The ease with which the influence of the differentiation centre is interrupted shows that it is not transmitted by a diffusing chemical. Seidel came to the conclusion, in fact, that the activity of the centre is fundamentally mechanical, and consists in a contraction of the yolk, which leaves a space into which the cells of the blastoderm migrate, thus forming the thickened region which becomes the germ-band. This contraction

normally begins in the middle of the differentiation centre, and spreads out in each direction from there; a comparatively slight mechanical abnormality, such as is caused by a loose constriction, will either inhibit the spread of the wave of contraction, or split into two. Thus the central yolk mass of the egg, which might at first sight be taken to be a merely passive supply of nutrients, is actually not so at all, but by its contraction plays an essential role in the epigenetic system which gives rise to the embryo.

After the action of the differentiation centre is completed, the various regions of the embryo seem to be fairly definitely determined, and only very slight regulation remains possible.

In the period since Seidel carried out his pioneer work, a number of other insect eggs have been found to follow a more or less 'indeterminate' type of development; that is to say, they are capable of some degree of regulation, and provide evidence of a sequence of epigenetic processes.

In the Coleoptera (beetles) it was shown long ago that there are special 'pole cells' at the posterior end of the blastoderm, and that if these are killed, no reproductive cells are produced by the embryo. In other respects, however, some regulation is possible. In the mealworm *Tenebrio* (Ewest 1937), there is a first stage which lasts from fertilisation till the sixth cleavage (sixty-four nuclei). If, during this stage, a posterior region (about 20 per cent of the total length) is removed or cauterised, the movement of the nuclei into the cortex ceases and not even a beginning of development occurs; but if a similar defect is made after the 64-nuclei stage, blastoderm formation proceeds although no embryo will develop. Thus this posterior region must exert some action in the very early cleavage stage, and it therefore seems to be similar to a formation centre, although it does not seem to require activating by the presence of a nucleus, as does that of *Platycnemis*.

In another beetle, *Leptinotarsa* (the 'Colorado beetle'), Haget (1953), in a very thorough and technically accomplished study, could discover no evidence for the existence of a formation centre active in the early stages. He is inclined to doubt whether Seidel's evidence in *Platycnemis* really proves the existence of such a centre, but in this he seems perhaps unduly sceptical.

Most recent workers have entirely confirmed the existence of a differentiation centre, lying further anteriorly, and operating some time after the beginning of development. Moreover our knowledge of the successive steps in the action of this centre has been greatly extended recently, particularly by the work of Bock (1942) on the neuropteran *Chrysopa*, Haget (1953) on *Leptinotarsa*, and Krause (1953) on the grasshopper *Tachycines*. All these forms give evidence of a series of inductive inter-

actions between the various germ layers, and thus suggest similarities between insect development and the phenomena which we shall find in the development of the vertebrates. According to the available information, there are certain differences in the epigenetic reactions in the various forms, but it seems not improbable that these may tend to disappear as the territory opened up by the recent pioneering work becomes more fully explored.

In all three forms, *Chrysopa*, *Leptinotarsa* and *Tachycines*, the differentiation centre begins to be active in the anterior part of the germ-band, which will later develop into the prothoracic region. Its presence is demonstrated by the fact that parts of the embryo removed from contact with the centre do not continue differentiating. Haget shows that in *Leptinotarsa*, the centre is at first localised in a small region, and gradually spreads in all directions (Fig. 8.6). In *Tachycines*, Krause finds that the first

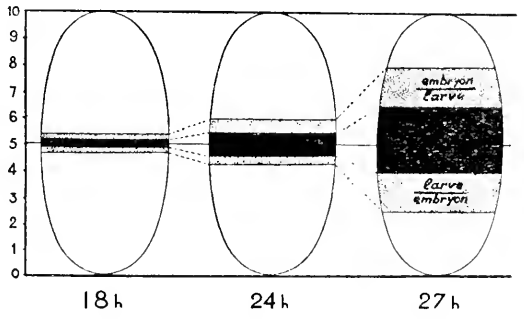


FIGURE 8.6

The increase in size of the 'Differentiation Centre' in *Leptinotarsa*, from 18 to 27 hours of age. If the embryo is cut in half through the black region, both parts develop partial embryos; if the cut is in the dotted region, the larger part forms a partial embryo and the remainder a germ-band which fails to develop further; while if the cut is in the white areas, only the larger part shows any signs of development. (From Haget 1953.)

result of the action of the centre is a tendency for the mesoderm to be invaginated. If, following injury, the mesoderm does not form, the ectoderm fails to develop; and Krause concludes that it is the mesoderm which endows the ectoderm with the capacity to differentiate. He points out, however, that the manner in which the ectoderm develops (i.e. the organs it forms) is not dependent on the nature of the mesoderm underlying it. In fact, once the ectoderm has been set going, the main inductive influence goes the other way, the character of the ectoderm determining what type of mesoderm shall be produced. Bock and Haget quite independently also obtained clear evidence for this induction of mesodermal tissues by the

ectoderm, in *Chrysopa* and *Leptinotarsa*. They both found, however, in contradistinction to Krause, that the ectoderm could self-differentiate histologically from an early stage quite independently of the presence of mesoderm, although in *Chrysopa* its morphogenesis is abnormal if it is not underlaid by mesoderm. Haget in particular has studied the gradual acquisition by the various regions of the ectoderm of the capacity for self-differentiation, and the accompanying loss of its ability to regulate. He has shown that the process is dependent on an influence which spreads from the differentiation centre through the sheet of ectoderm; he speaks of it as a process of 'intra-dermal induction', and it may be compared with the 'individuation' or 'regionalisation' by which various organs become localised within the sheet of invaginated mesoderm in the vertebrates (Fig. 8.7).

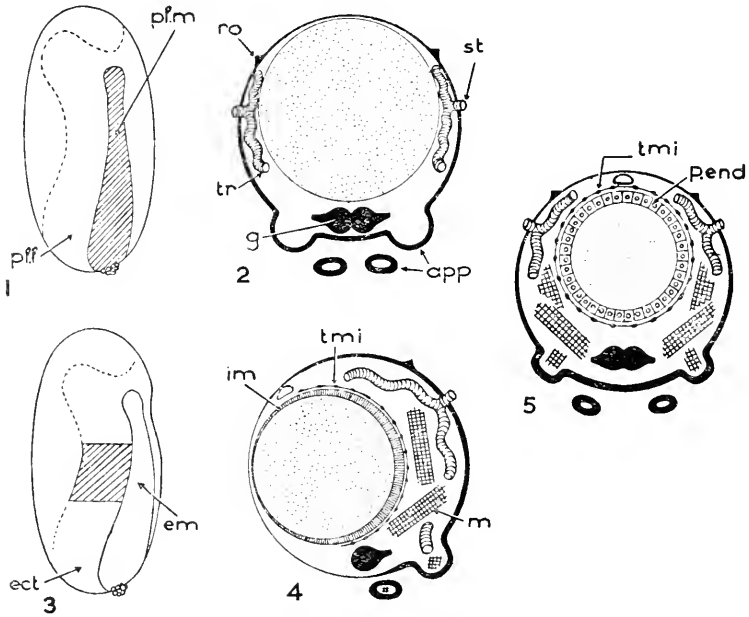


FIGURE 8.7

Results of cauterisation of the endo-mesoderm or ectoderm in *Leptinotarsa*. Figure 1 shows the whole median plate (*pl.m.*) of the germ-band destroyed. In the resulting embryo (Figure 2) the ectodermal organs are present (*tr.* tracheae, *g.* ganglion, *app.* appendages) but there are no mesodermal or endodermal tissues. In Figure 3 one of the lateral plates of the germ-band (presumptive ectoderm) has been cauterised. The embryo formed (Figure 4) lacks ectodermal organs in the cauterised region, and the endodermal and mesodermal organs are also absent where the ectoderm is defective. Figure 5 shows a section through a normal embryo (*p. end.*, endodermal lining of gut, *m.*, muscle, *im.*, midgut, *tmi.*, mesodermal tunic of midgut). (After Haget 1953.)

It is a most remarkable fact that in the insects it is the ectoderm which takes the lead in the determination of development, while in vertebrates this function belongs to the inner layers, endoderm and particularly mesoderm. Whether this has any connection with the 'upside-down' morphology of the adult insect as compared with the vertebrate (ventral nerve cord instead of dorsal) is an intriguing question. One might, perhaps, think that it was more likely that conditions in the insects should be similar to that in annelids; but again, in *Tubifex* as we have seen, the development of the ectoderm is dependent on influences from the mesoderm (p. 99). There seems to be no close parallel in other groups to the situation found in the insects.

Haget has also evidence of a later inductive process, by which the mesoderm, after submitting to the influence of the ectoderm, itself induces the differentiation of the gut endoderm.

The Hymenoptera (Fig. 8.8) are a group which can be considered as intermediate between the indeterminate and fully determinate types,

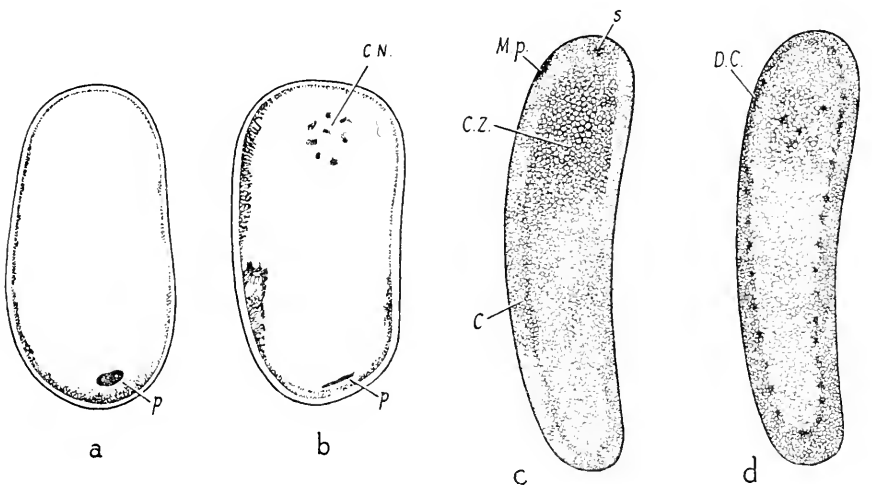


FIGURE 8.8

a, *b*, Longitudinal sections through eggs of the ant *Camponotus*: *a*, shortly after laying, with cortex more or less evenly spread over the whole surface except for a slight thickening at the posterior end; *b*, 6–12 hours later, showing the differentiation of regions within the cortex. *P* is the collection of granules which will pass into the pole-cells (future germ-cells); *C.N.*, cleavage nuclei. (After Reith 1931.)

c, *d*, Longitudinal sections of eggs of the bee, *Apis*: *c*, just after laying; *d*, stage with 512 nuclei. *C*, cortex, *C.Z.*; central zone; *D.C.*, Differentiation Centre; *M.p.*, maturation plasma; *S*, sperm nucleus. (After Schnetter 1934*a*.)

although somewhat nearer the latter. The main studies in this group have been on an ant, *Camponotus* (Reith 1931), and the bee, *Apis* (Schnetter 1934*a*, *b*). In the former, the cortex is at fertilisation more or less uniform in thickness over the whole surface of the egg, although perhaps slightly thicker on the ventral side. As early as the stage of nuclear cleavage, however, it becomes differentiated into five zones, by the flow of internal cytoplasm to particular parts of the surface. This earliest stage of differentiation is suppressed if the posterior end of the egg is destroyed by cauterisation, which perhaps indicates that it is controlled by something analogous to a formation centre. The most important of the zones is that from which the germ-band arises, which may be compared to the differentiation centre. Cauterisations in the second half of the cleavage period allow the differentiation of the zones to proceed, but the germ-band zone may be shifted somewhat from its normal position. After the cortical zones have once been fully developed, no further regulation seems to be possible; both the formation centre and the differentiation centre have finished their work before the blastoderm is developed. (It is worth remarking that two of the cortical zones in the ant are concerned with the uptake of symbiotic bacteria, which seem to be always present in the egg, and which finally arrive in the midgut; their function is obscure.)

In another hymenopteran, the bee *Apis*, development is even more precocious, since there are clearly marked cytoplasmic regions in the egg before fertilisation. The cortex is thicker on the ventral side, and in the anterior of this side there is a special collection of cytoplasm in which the maturation of the egg nucleus occurs. Slightly posterior to this is the region of the greatest diameter of the egg, and in this neighbourhood the cortex is thicker and there is more internal cytoplasm mingled with the yolk. A column richer in cytoplasm and poorer in yolk extends down the whole centre of the egg. This structure of the egg clearly affects the migration of the cleavage nuclei, which move into an elongated oval. The shape of this is not quite symmetrical, since the nuclei reach the surface first in the anterior ventral region, which can be considered as the analogue of the differentiation centre. As in *Platycnemis*, this seems to be the focus of a contraction of the internal material of the egg, but in this case the contraction is concerned not so much with the formation of a simple thickening of the blastoderm, but rather with the infolding of the inner layer, which occurs at a slightly later stage. There is in the bee as yet no evidence for the operation of a formation centre preceding the differentiation centre, but if small portions of the anterior part of the egg are removed by cauterisation, the differentiation centre shifts slightly posteriorly and complete but dwarf embryos are formed. By the early

blastoderm stage, the position of the centre is fixed, but a small amount of regulation is still possible within individual organs; that is to say, if an egg in this stage is ligatured, each end forms only part of an embryo, but each of these parts consists of a certain assemblage of complete regions, i.e. a complete anterior head, or complete jaw region, complete thorax or complete abdomen. At a slightly later stage, even this degree of regulatory power is lost, and ligatured eggs develop in a completely mosaic way, producing part thoraces or part abdomens exactly according to the location of the ligature.

In fully determinate eggs, such as those of Diptera, almost completely mosaic development occurs from the very earliest stages, and after cauterisations or ligaturing, no sign of regulation is found. Dwarf embryos have, however, been produced by strong centrifuging which shifts the cortex (Pauli 1927) and it appears that it is in the cortex that the forerunners of the embryonic organs are located. Small drops of the internal cytoplasm can in fact be removed (by pricking and allowing them to exude) without causing abnormalities in development (Howland and Sonnenblick 1936) (Fig. 8.9).

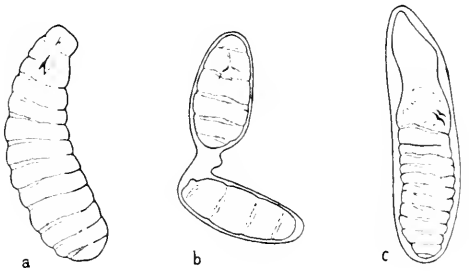


FIGURE 8.9

a, Normal embryo of the dipteran fly *Calliphora; b*, embryo formed after constriction of the egg at the 16-cell stage, showing no sign of regulation; *c*, shortened and partially regulated embryo following strong centrifugation of newly fertilised egg. (After Pauli 1927.)

A little information on the epigenetics of the dipteran *Drosophila*, which is so important for genetical studies, is beginning to be produced by less conventional methods. Thus Yao (1950) has investigated histochemically the distribution of acid and alkaline phosphatase, and finds that the latter makes its appearance in the future thoracic region of the germ-band some time after the mesoderm has invaginated, and gradually spreads throughout the ectoderm, by a process which he compares with the

infective transmission of pigment formation in guinea-pig skin (p. 397). This reminds one strongly of the Differentiation Centre and its behaviour as described by Haget in *Leptinotarsa*.

Another method which has recently been employed is the application of ultra-sonics for the purpose of stirring round the internal contents of the egg (Selman and Counce, 1953, 1955). In cleavage stages this treatment may cause many types of cytoplasmic disturbance. If it is applied when the nuclei have arrived at the surface, preparatory to forming a cellular blastoderm, it may prevent the gastrulation movements, so that the tissues differentiate fairly normally but in the positions which they have before gastrulation occurs; or it may shift around the various cellular regions, in which case the organs are later found in quite unusual positions. In the latter case, there is usually some abnormality or deficiency in tissue differentiation; in particular the hypodermis tends to be badly developed. There are suggestions that some of these abnormalities of differentiation are consequences of disturbances of inductive relationships, but in most cases the evidence is not clear. Selman and Counce find, however, that gonads may develop in quite unusual parts of the body to which the germ cells have been transported, and argue that these cells induce the mesoderm near them to develop into the gonad sheath. (In *Leptinotarsa* Haget [1952] finds that the gonad mesoderm can differentiate in the absence of the germ-cells, and the same is probably true of *Drosophila* [Aboim 1945] though here the evidence is not entirely convincing; however even if it is accepted, this would not disprove the suggestion that the germ-cells can also exert an inductive influence.)

The most important aspect of the embryology of Diptera, however, is not its analysis by the operative methods of normal experimental embryology, but the opportunity provided by the enormous wealth of genetical material available in *Drosophila* to study the action of genes in early development. The pioneer in this work was Poulson (1945, 1950), and the most recent work that of Ede (1954) and Counce (1955). The principle which has been followed is to study the development in cases where the genetic situation causes death before hatching (in which case one can be certain that something fairly drastic is going on). A considerable number of factors have now been investigated, and the most important general points emerging seem to be the following.

Genes may be active at a very early stage of development. Poulson (1945) found that the absence of the whole or the greater part of the *X* chromosome causes disturbances of the cleavages and the migration of the cleavage nuclei. Ede found that certain sex-linked genes, which may be point-mutations, or may be very small deficiencies, may have the same

effect; thus it is not only large amounts of chromosome, but on the contrary individual genes which are active at this stage.

A considerable number of genes are found to affect the process of gastrulation, which involves extensive movements of the blastoderm. This is clearly a delicately balanced process, which can easily go wrong; it is an 'epigenetic crisis', that is, a time at which minor abnormalities which have occurred earlier suddenly produce far-reaching and drastic effects.

Certain genes cause considerable abnormalities in tissue differentiation. These seem to be of two kinds; general retardation or impairment of differentiation, the cells remaining rather embryonic in character; or the switching of cells which should develop into one type of tissue into some other of the characteristic types. An example of the latter effect is the observation that in several cases an abnormally large proportion of the hypodermis develops into neural tissue, leaving little or none to form skin. This might be the result of a disturbance of an intra-dermal inductive process within the hypoderm, of the kind postulated by Haget (p. 130).

These facts make clear the importance of chromosomal genes in the early developmental processes. Counce (1954) has recently studied in detail some stocks in which the importance of the cytoplasm becomes obvious. These are 'female-steriles', that is to say, races in which females homozygous for a given gene produce eggs which do not develop properly (see also Beatty 1949). This failure must be due to abnormalities in the cytoplasm formed under the influence of these genes in the ovarian tissues of the mother. In one of the genes studied, deep orange, the abnormality is similar to the first kind mentioned above, in that it is manifested in the early cleavage divisions. Another, fused, affects some of the elongation movements concerned in gastrulation (Fig. 8.10), while a third brings about an arrest of differentiation during a certain period of later embryogenesis. There is thus a considerable variety of processes which such cytoplasmic factors may influence.

The nature of the cytoplasmic factors is not at all clear. They may perhaps be 'plasmagenes' (p. 387), that is to say, have some power of reproduction; but it is unnecessary to make this assumption, since no overall growth has occurred by the time they begin acting. A very interesting fact, however, is that their adverse effects can be to a large extent overcome by the normal allele of the locus. If eggs from a female homozygous for one of these female-steriles (e.g. fused) is fertilised by sperm carrying a normal *X* chromosome, complete development may occur, and even if it does not do so, the egg develops more normally than otherwise. Some alleviating effect of this kind, though less in degree, is found even

when such eggs are fertilised by chromosomes bearing a *Y* chromosome (which, if they could develop fully, would produce males). In this case the alleviation is probably caused by supernumerary *X*-bearing sperm, which enter the egg cytoplasm, but later degenerate, since they do not unite with the egg nucleus; it is known that in *Drosophila* it is common for five or six sperm to penetrate the egg.

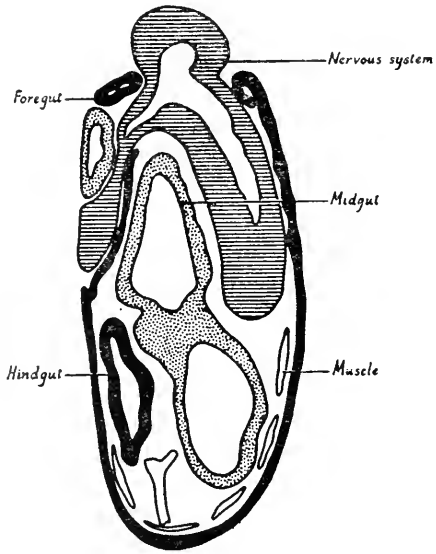


FIGURE 8.10

Diagrammatic longitudinal section of *Drosophila* embryo developed from an egg of a *fused* mother mated to a *fused* male: displacement of organs due to faulty gastrulation movements. (From Counce 1955.)

We are thus beginning to get, in *Drosophila*, some insight into the interaction of genes and cytoplasm in early development, a subject which is obviously of the greatest importance, but in which much remains to be done. The study of hybrid merogons in Amphibia (p. 358) and echinoderms belongs to the same general sphere of interest, but in those organisms we cannot, as yet, investigate the effects of individual genes.

3. *The transformation of the embryo into the adult.*

All insects, as they grow, pass through a series of moults, in which the external cuticle is shed and a new cuticle formed around the enlarging insect. As the moults proceed there are changes in the organisation of the

animal, which finally becomes an adult. In some types, these changes are fairly gradual ('hemimetabolous' insects with an incomplete metamorphosis), in others there are first a series of larval stages in which little alteration occurs except increase in size, but these are followed by a rather sudden radical reorganisation by which the adult is produced ('holometabolous' insects with a complete metamorphosis). The period during which the metamorphosis occurs is known as pupation, and the pupal form usually differs considerably both from the larval and the imaginal phases of the life-history.

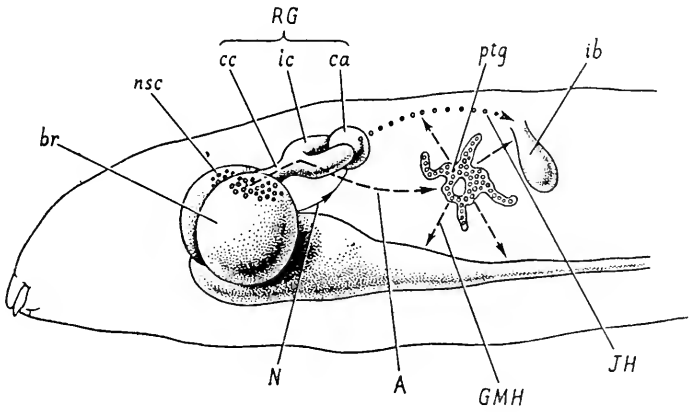


FIGURE 8.11

Diagram of the hormonal control of moulting and metamorphosis in insects. The letters above refer to the relevant larval organs: *br.* the brain; *nsc*, the neurosecretory cells; *R.G.*, the ring-gland (in *Diptera*) consisting of *cc*, the corpus cardiacum, *ca*, the corpus allatum, and *lc*, the lateral cells, which probably function as the prothoracic gland, which in other forms lies some distance from the corpus allatum, and is indicated as *ptg*; *ib* is an imaginal bud. The letters below refer to the active principles: *N*, the nervous connection to the corpus allatum; *A*, the activator passing from the neurosecretory cells to the prothoracic gland; *GMH*, the growth and metamorphosis hormone given out by this gland; *JH*, the juvenile hormone produced by the corpus allatum.

Both the moulting of the larva and its metamorphosis to the adult are controlled by hormones (Reviews: Seidel 1952*a*, Wigglesworth 1954, Bodenstein 1954). There are at least two main hormones involved, and probably more. The anatomical structures in which the hormones are produced are not always easy to homologise from one group of insects to another, so that the details of the story are complex; only a general summary can be given here.

The two hormones which are most fully authenticated are, firstly, a 'growth and moulting hormone', which has the effects suggested by its name, and secondly a 'juvenile' hormone. The effect of the latter is to *prevent* the moulting larva from developing into the pupa or adult; metamorphosis is inhibited until the concentration of juvenile hormone, which falls throughout larval life, has sunk low enough. The growth and moulting hormone seems always to be secreted by a gland located in the thoracic region, usually known as the prothoracic gland. The activity of this gland is, however, itself stimulated by an 'activating' substance. This is, in many cases, formed by certain neurosecretory cells in the brain; it is sometimes transmitted along the nerves, and in particular into an annexe of the brain known as the corpus cardiacum, from which it may be released into the haemolymph to reach eventually the prothoracic gland. The juvenile hormone is secreted from another organ, known as the corpus allatum; and this again may be activated by influences from the brain, which in this case are probably nervous in nature.

In the higher Diptera, such as *Drosophila*, the interactions between these organs are made more confusing by the fact that they all lie very close together. The main hormones are produced in an organ known as Weissman's ring, or the ring gland. This is closely attached to the upper part of the brain, that is to the region of the neurosecretory cells. The ring gland itself is complex; the part nearest the brain corresponds to the corpus cardiacum, that furthest away to the corpus allatum, while the lateral parts probably function as the prothoracic gland (Fig. 8.11).

The growth and moulting hormone is produced periodically towards the end of each instar throughout the whole of larval life. If the source of the juvenile hormone is removed from a young larva (e.g. by extirpating the corpus allatum) a premature metamorphosis occurs, giving rise to a dwarf pupa or adult (Fig. 8.12). On the other hand, if corpora allata from young larvae are transplanted into a larva ready to metamorphose, it is caused to undergo an extra larval moult instead and only finally metamorphoses a stage later, forming a giant. Moreover, by removing the source of metamorphosis hormone when it has begun but not completed its secretion, or by implanting corpora allata from earlier stages, it has been possible to obtain abnormal balances between the two hormones and thus to provoke partial metamorphoses, which produce hemipteran individuals intermediate between nymph and adult, and lepidopterans intermediate between larva and pupa.

In some insects, the life-cycle includes not only moulting and metamorphosis, but also a period of complete standstill, a so-called diapause. It is often in the form of such a resting stage that the animal passes the

winter. The diapause may occur either during embryonic development in the egg, or during the early part of the pupal period. The peculiar physiological conditions which enable the animal to survive in a state of arrest have aroused a good deal of interest, and a fair amount has been discovered about them in certain cases. In the *Cecropia* silkworm (Lepidoptera) Williams (1951) has shown that the diapause, which occurs in early

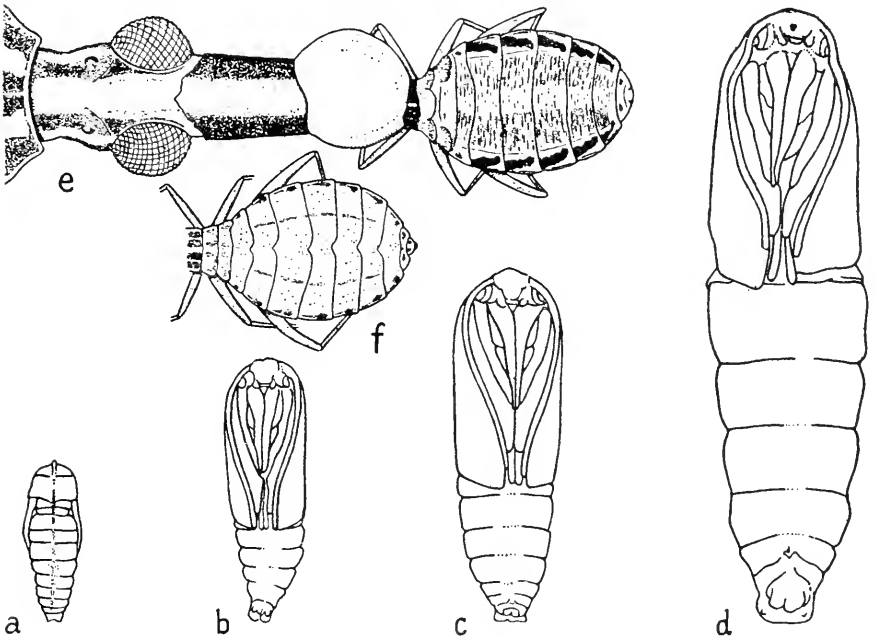


FIGURE 8.12

Figures *a* and *b* are dwarf pupae of the Wax moth, resulting from the removal of the corpus allatum (source of the moulting hormone) from third and fourth instar larvae; *c* is a normal pupa, and *d* a giant one produced by implanting an extra corpus allatum from a young larva into one which had already reached the stage at which it would normally pupate. (After Piepho 1943.) *e*, a precocious adult of the bug Rhodnius produced by joining a 1st-stage larva to a larve undergoing the final moult; *f* is a normal 2nd-stage larva for comparison. (From Wigglesworth 1934.)

pupal life, is under hormone control. It does not seem to be quite clear how the diapause is initiated; but once the animal has passed into diapause, it remains in that condition until the brain has been cooled for a certain length of time, and then re-warmed. After this alternation of temperatures, the brain is able to emit a hormone which activates the prothoracic gland, and this in its turn produces a second hormone which starts off the development of the pupa into the adult. The effect of the

prothoracic hormone can be analysed to some extent in biochemical terms; it causes profound changes in the cytochrome enzymes which are concerned with respiration in the pupal tissues. This brings about a complete change in the metabolic system of the insect, and it is clearly as a result of this change that the development of the various organs is once more able to proceed.

It is perhaps worth emphasising the obvious fact that the metamorphosis and diapause hormones do not determine what type of development any particular tissue will undergo, since they affect equally all the different rudiments. They act as what have been called 'realisers', which make it possible for potentialities to become actual, but they are not 'determiners', which could change the characters of the reacting tissues. The change they bring about is, at least in the Hemimetabola, to be compared with a modulation (p. 14) rather than a determination. It can be to some extent reversed, since if an adult bug is provided with large amounts of juvenile hormone, it may moult again and the larval characters reappear (Wigglesworth 1948*a*, *b*).

4. *The determination of imaginal characters.*

In insects with complete metamorphosis, such as the Diptera, the future imaginal tissue is present during larval life in the form of separate pockets of cells, the so-called imaginal buds. These originate from the hypodermis of the embryo, which is part of the ectoderm. In the early stages of larval life, there is some variation in the readiness with which the different buds react to a given concentration of metamorphosis hormone, and it appears that they undergo, at slightly different rates for different buds, a process of maturation by which they acquire an increasing competence to respond (Bodenstein 1943, 1950). There has been considerable debate as to when these buds become determined in their developmental fate. By irradiating *Drosophila* embryos with ultraviolet, Geigy (1931) was able to produce purely imaginal defects in animals whose larvae had developed perfectly normally. This occurred only when the irradiation was given about seven hours or more after laying, at a time when the embryo is well on the way to formation. It therefore appears that, long after the period of embryonic determination (which is extremely precocious in Diptera) there is a second period when the imaginal buds are determined. Lüscher (1944) has recently produced somewhat similar evidence concerning the Lepidoptera. Evidence tending in the same direction has been brought forward by Gloor (1947) who found that by ether treatment of the young *Drosophila* embryo he could cause the metathoracic imaginal bud to develop in the way characteristic

of the mesothoracic one, a result similar to that produced by the gene *bithorax*.

It was for some time thought that the determination of the imaginal buds which occurs in mid-embryonic life was the final step which fixed the fate of each part, converting the buds into a rigid mosaic. For instance Bodenstein (1941) found no signs of regulation when limb-buds from third instar larvae were halved. However, Waddington (1942*a*, *b*) showed that the determination is by no means final even in larval stages (Fig. 8.13). If early third instar larvae are given a heavy dose of x-rays,

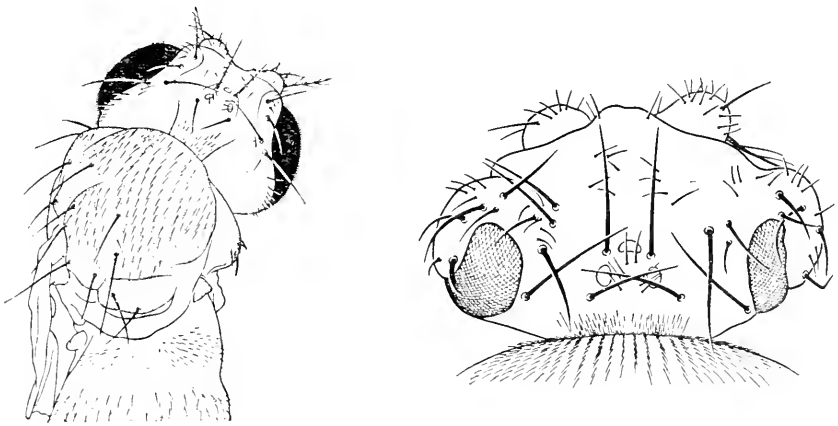


FIGURE 8.13

Regulative development in *Drosophila*. On left, a *vestigial* fly in which one mesothoracic bud has failed to evert and the other produced considerably more than half a thorax. (From Waddington 1953.) On right, conversion of eyes into palps following x-raying of the late larva. (From Waddington 1942*b*.)

many of the imaginal bud cells are killed, and those that remain may produce duplicated organs, or even something quite foreign to their normal fate (e.g. eyes in place of antennae or vice versa). Various authors then found that when the imaginal buds of the larva are cut into fragments which are allowed to develop in isolation, their behaviour is not strictly mosaic. Hadorn and Gloor (1946) showed that the female genital bud behaved like a series of overlapping fields (Fig. 8.14), and Hadorn, Bertani and Gallera (1946) found that in the genital bud of the male considerable reorganisation and regulation of these fields is possible. Vogt (1946*a*) obtained very similar results with the eye-antennal bud. The same author (1946*b*) studied the development of eye-antennal buds of flies homozygous

for the gene aristopedia. This causes part of the antennal bud to develop into a leg instead of an antenna; and it was found that the amount of the bud which is diverted into this abnormal channel of differentiation can be altered by the temperature to which it is subjected during the third larval instar. One must conclude that the determination of the imaginal character is still very labile until at least the time of pupation. Shatoury (1955)

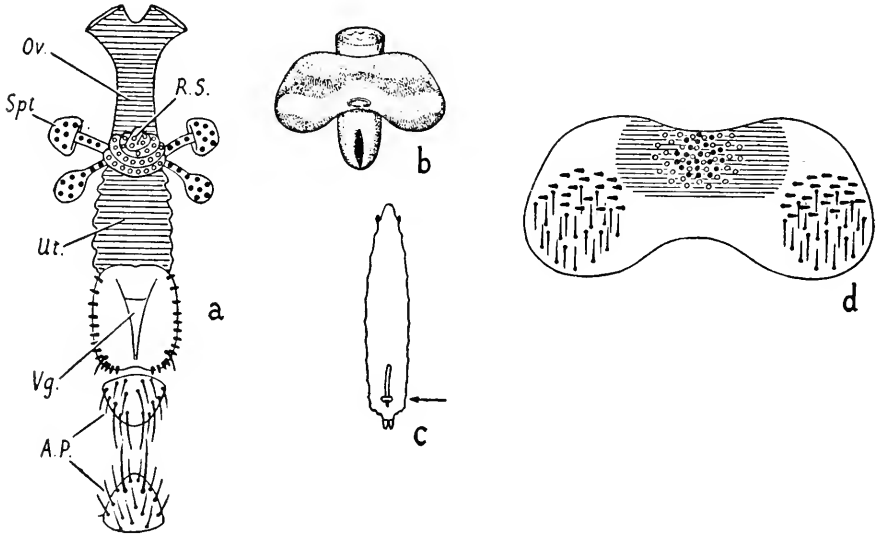


FIGURE 8.14

The female genital bud of *Drosophila: a* shows the genital organs of the adult; *Ov*, oviduct, at the top the two branches which continue to the ovaries have been cut through; *R.S.* receptaculum seminalis; *Spt.*, spermatheca; *Ut.*, uterus; *Vg.*, vaginal plate; *A.P.*, anal plates. The larval bud, lying across the intestine, is shown in *b*, and its position in the larva in *c*. The results of transplantations of fragments of the bud are summarised in *d*, which shows the overlapping fields from which the various organs are formed (shading corresponding to those of Figure *a*). (After Hadorn and Gloor 1946.)

argues, on the basis of aberrant types of development found in certain mutant stocks, that the essential features of the imaginal buds are determined by influences from the mesoderm which migrates into the buds during the third instar. Thus the determination of the buds which occurs in the embryo can only be of a preliminary and tentative kind.

Even during the period of pupation, some degree of regulation is possible to the various imaginal buds. Waddington (1953) and Pantelouris and Waddington (1955) found that if one of the mesothoracic buds is

removed, or if it fails to evert, the bud on the other side may regulate so as to form more than the half-thorax which is its normal fate.

Non-mosaic behaviour of a rather different kind is also exhibited by the gonads and genital ducts during the pupal period. Dobzhansky (1931) first pointed out that if the testes of *Drosophila simulans*, which are normally spiral in shape, fail for some reason to make contact with the ducts which develop from the genital disc, then the spiralisation does not occur, and the testes remain ovoid. Stern (1941) studied the matter in detail, and showed clearly that the ducts induce in the testes the asymmetric growth which leads to the assumption of a spiral shape. In some species of *Drosophila*, the testes normally grow more or less equally in all directions, and thus remain ovoid; and Stern found that if the larval testis of a species which should develop a spiral gonad becomes attached to the genital duct of a non-spiralising form, then it also fails to become spiral. This is one of the rather few cases in which a species difference is brought about by a difference in an inductive action rather than by being dependent on the nature of the competence of the reacting material. But, as Stern points out, we are dealing here with the transmission of an asymmetric growth stimulus and not with the evocation of histological type of tissue (Fig. 8.15).

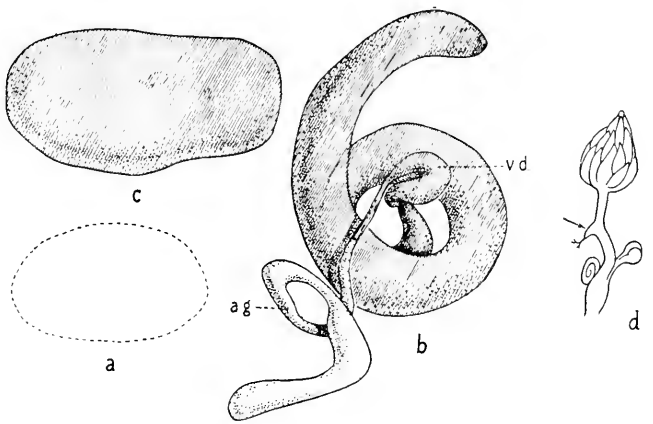


FIGURE 8.15

Interaction of gonads and genital ducts in *Drosophila melanogaster* pupae: *a* shows the outline of the testis at the time it becomes attached to the male duct; *b*, the normal coiled form it assumes; *c*, the uncoiled form resulting from failure of attachment. (From Stern 1941.)

Figure *d* shows the adult female genital tract in a fly from which one larval ovary was removed; the oviduct (arrow) to which no ovary becomes attached fails to elongate. (From Pantelouris 1955.)

The reaction between the testis and the ducts is not all in one direction, since pigmented cells migrate out from the testis sheath on to the duct, whose colouration is thus dependent on the kind of testis with which they come in contact (Stern and Hadorn 1939). A more drastic effect of the gonad on the associated duct occurs in females, where Pantelouris (1955) has shown that the lateral branch of the oviduct does not elongate unless it makes contact with the ovary. The ovary itself seems to be relatively independent in its growth, and can, though rather rarely, attain its normal size even when not attached to any genital duct; this may occur even in a male host.

SUGGESTED READING

Most of the original literature on experiments on embryos is in German; Seidel 1936, 1952*a* recommended; Haget 1953 is a fine original paper (in French); Wigglesworth 1947 summarises some of the literature; see Poulson 1945 for chromosomal control.

For pupal stages, Hadorn 1948*b*, Waddington 1942*a*.

For pupation hormones, Williams 1951, Wigglesworth 1954, Seidel 1952*a*.

CHAPTER IX

THE VERTEBRATES: THE AMPHIBIA AND BIRDS

THE GROUP of vertebrates contains a variety of types which are sufficiently diverse to exhibit most of the important principles of embryology, but are not so bewilderingly various as to obscure the fundamental plan of which they are all modifications. They are therefore peculiarly suited for comparative study. Moreover, the rather large size of many vertebrate eggs has made them favourite objects for experimental analysis, and our understanding of the epigenetics of the group is at least as great as that of any of the invertebrate phyla. This is particularly true of the Amphibia, and only slightly less so of the birds. These two groups have for long been classical teaching material, since frogs' and chickens' eggs are some of the easiest to obtain for students' use. The discussion of vertebrate development in this book will be largely based on these same two objects, although as an amphibian type, the newt's egg will be referred to perhaps more often than that of the frog, since, although they are basically similar in the characters of interest in an elementary account, the former shows these features in a somewhat clearer way; moreover, for technical reasons connected with ease of manipulation, it has proved more favourable than the frog egg for experimentation. It is only after the early development of these two types has been described and discussed that we shall turn to consider, more shortly, the embryology of the other vertebrate phyla.

Detailed descriptions of the development of Amphibia are to be found in many general embryological textbooks, particularly Dalcq and Gerard (1935). For the chick there are several special monographs. Patten (1950) and Huettner (1949) are good descriptive texts, the figures in the latter being particularly clear; Hamilton's (1952) revision of Lillie is the most complete descriptive monograph, but tends to neglect non-American work; Waddington (1952*a*) deals mainly with the early stages of development, and with experimental studies. Details of most microsurgical techniques are given by Hamburger (1942) and Rugh (1948), but neither of them deals with organiser grafts in birds.

1. *From the unfertilised egg to the formation of the blastula*

(*a*) *The Amphibia*

The fully grown frog's or newt's egg is a fairly large spherical cell, some 2 or 3 mm. in diameter. It is usually seen after being extruded into the

water; normally the eggs are fertilised either as they leave the female's body (in frogs) or by sperm which the female has taken up into her cloaca (in newts). The egg within the ovary and oviduct is surrounded by a viscid layer of jelly, which swells on contact with water into a thick protective covering; in newts each egg is enclosed within a separate capsule, but in frogs a whole clutch of eggs coheres into a single mass within which the individual eggs are scattered.

Amphibian eggs contain moderate quantities of yolk; much more than non-yolky types such as echinoderms or ascidians, much less than the extremely yolky birds' eggs. The yolk is present in the form of small platelets or ovoid granules, and, although scattered throughout the whole egg, is more concentrated at the vegetative end where the granules are also larger in size. Near the opposite, animal, pole is a large germinal vesicle filled with clear sap. The heavy load of yolk granules makes it rather difficult to distinguish different regions of cytoplasm, such as those so well shown in the ascidians, and it is only recently that the earliest phases of development are becoming clear (Reviews: Pasteels 1951; Dalcq 1950*b*). There is, for instance, always a peripheral zone or cortex which contains little yolk; the animal half of it usually carries a considerable number of pigment granules, which make up a dark animal cap which is very clear in the frog; the outermost layer of all is a relatively impervious, very extensible membrane, the 'coat' (Holtfreter 1943*a*). In the interior of the egg, it is usually possible after the germinal vesicle has broken down to distinguish a clearer animal plasm, in which the yolk granules are smaller and more scattered; this appearance may be due to the admixture of the nuclear sap (Fig. 9.1). In some forms (the frog and axolotl) there is also a darker 'marginal plasm' which lies in a circle fairly close under the surface just above the equator, and in the frog there is another, central, region with small yolk platelets (Pasteels 1951).

The pattern of cytoplasmic regions in the unfertilised egg is thus radially symmetrical and shows no obvious sign of the future dorso-ventral plane of symmetry. An indication of this plane does, however, appear fairly soon after fertilisation in many amphibian eggs. The first apparent result of fertilisation is a slight lifting-off of the inner or vitelline membrane from the egg, which thus becomes free to revolve within its jelly capsule and lie in its natural position with the heavy yolk-laden vegetative pole downwards; the swing round into this position takes only a few minutes. Soon afterwards, in the frog and some other species, a 'grey crescent' appears on one side, lying between the dark animal hemisphere and the pale yolky vegetative end. This is destined to play a fundamental part in later development. Its fate cannot be followed without special methods,

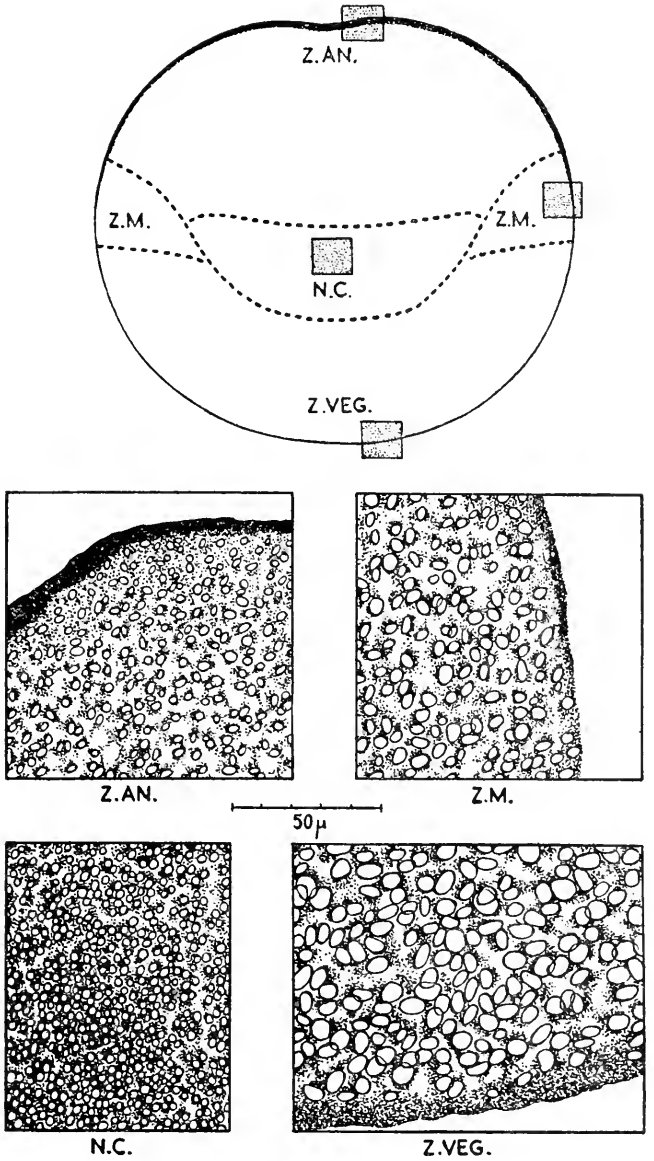


FIGURE 9.1

Oöplasmic regions of the unfertilised egg of *Xenopus*. The main regions are: the animal region (*Z.AN.*); the marginal zone (*Z.M.*); the central region (*N.C.*) and the vegetative region (*Z.Veg.*). The upper drawing shows the location of these in a transverse section, while the lower drawings illustrate the nature of the cytoplasms. (From Pasteels 1951.)

since as the egg cleaves up into many cells, the even colour of the animal pole becomes broken up by the cell boundaries so that it merges into the paler tint of the crescent, which ceases to be recognisable. But we can apply the technique of vital stained marks (p. 158). A small spot of colour placed in the centre of the grey crescent is found eventually to lie in the dorsal midline of the animal, and, in fact, somewhere in its notochord. The grey crescent therefore marks the dorsal side, and, in its position, corresponds to the grey chorda-neural crescent which appears, at a somewhat later stage, in the ascidian egg.

The appearance of the grey crescent and the marking of the dorsal side is the first great step in embryonic development in the Amphibia. Naturally it is important to know its causal antecedents and its causal consequences; what brings it about, and what effect does it have? There is general agreement about the latter. After the grey crescent has appeared, every developmental performance of the egg is related to it. If, for instance, the egg is cut in half (which can be done even before the first cleavage by putting the egg into a loop of fine hair which is slowly pulled tight (Fig. 9.2), then only those halves which contain some grey crescent material will develop any of the main embryonic tissues, such as neural system, notochord, somites, kidney, etc.; ventral portions of the egg which have no crescent material, usually form only skin and disorganised mesoderm and endoderm not recognisable as any definite tissue, though Dollander (1950) has recently shown that in some cases a certain degree of regulation occurs and the ventral parts also produce a little neural tissue and axial mesoderm. The grey crescent, in fact, is the precursor of the 'organisation centre' of the gastrula, which, as we shall see, (p. 175) is the agent which causes the formation of the rest of the embryo.

The antecedents of the grey crescent are less well understood (Ancel and Vintemberger 1948, Pasteels 1951). Its position is certainly not completely fixed before fertilisation, since it is possible by suitable treatment to make it appear in any desired meridian of the unfertilised egg. If, for instance, an egg is fertilised with sperm brought on a needle to a given place on the surface, the grey crescent usually appears at or near the diametrically opposite side. Similarly, if the newly fertilised egg is held for some time so that one side of the yolky hemisphere is much higher than the other, the grey crescent usually appears on the higher side.

The experiments of the last paragraph demonstrate that the grey crescent is not fixed before fertilisation, or indeed much before it actually appears. But the same experiments also suggest that there is a pre-disposition of a certain plane to become the plane of symmetry. The experiments of tilting the egg, or of fertilising it in a definite place, do not *always*

determine the position of the crescent; the cases in which they fail are those in which they do not overcome the existing predisposition. Again, in normal development there seems to be a tendency for the sperm to enter the egg in a predetermined plane, so that its influence on the position of the crescent usually reinforces a prior condition. Finally, when newly

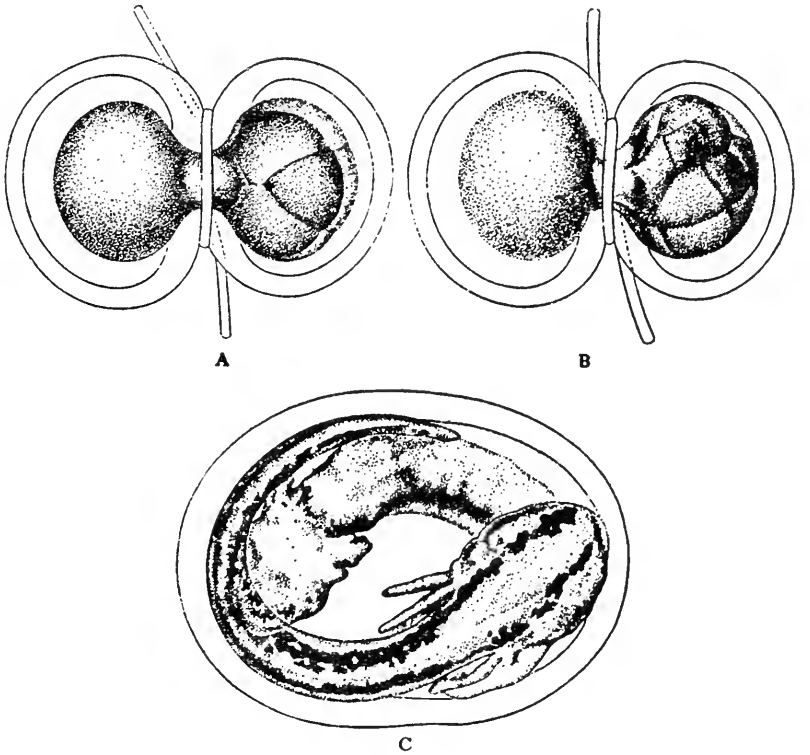


FIGURE 9.2

A newt's egg, in its jelly capsule, is constricted into a dumb-bell shape soon after fertilisation. *A*, cleavage occurs first in the portion containing the nucleus; *B*, after some time a nucleus passes through the stalk, after which cleavage begins in the other (left) portion also; *C*, both parts may give rise to a complete embryo. (From Schleip 1929, after Spemann.)

fertilised eggs are constricted into two halves with a hair loop, we find that certain halves, although they contain the nucleus, develop into featureless lumps exactly similar to those, derived from a later stage, which contain no grey crescent material; and this indicates that, even immediately after fertilisation, the crescent material is to some extent localised so that egg fragments may contain none, or too little, of it.

In recent times, Pasteels is the author who has studied in most detail the relation between the grey crescent and the various plasmatic regions of the egg (see his review, 1951). The internal contents of an amphibian egg are fairly fluid and if the egg is turned upside down and held with the vegetative pole uppermost, the heavy, yolky material from that end streams down and comes to lie against the cortex near the animal pole region. The grey crescent is not distinct enough in appearance always to be recognisable with certainty in such eggs, but as we have seen, in later development it gives rise to the blastopore and that structure, at least, is unmistakable. It is found that blastopores always appear at the edge of regions in which masses of yolky cytoplasm are in contact with the cortex (Fig. 9.4). At these blastopore regions invagination takes place and an embryo will eventually develop. Its cephalo-caudal polarity is determined by the gradient in yolk content, the future head end always originally lying nearest to the most concentrated mass of yolky cytoplasm. It is clear from these experiments that the position of the grey crescent is determined by the mutual relations of the yolky cytoplasm and the cortex.

In eggs which have been held upside down and in which drastic alterations of the internal structure have occurred, it is difficult to be certain of the nature of the interaction which takes place between the yolky cytoplasm and the cortex. In the normal egg Ancel and Vintemberger have shown that the formation of the grey crescent involves a movement of the cortical layer of that region towards the animal pole (Fig. 9.3*B*). This seems to carry up with it some of the underlying yolky cytoplasm which thus becomes thoroughly mingled with the marginal cytoplasm with its larger content of basophylic granules and mitochondria. It seems probable that it is this mingling of yolky and marginal cytoplasm which is the essential feature of the grey crescent. If shortly after fertilisation and before the appearance of the normal grey crescent an egg is tilted slightly so that the animal-vegetative axis makes an angle with the vertical, the yolky cytoplasm slides down into the lowest position and in doing so leaves behind it a sub-cortical layer which has much the same appearance as that of a normal grey crescent: and as we have seen it eventually develops into a blastopore. This experiment was one of the earliest to be carried out in amphibian experimental embryology. It was originally performed by Born in the 'eighties of the last century. It has recently been studied in detail by Pasteels (1951). Pasteels also shows what happens when the experiment is carried out slightly later, after the appearance of the grey crescent. In eggs rotated at this time the blastopore will, again, eventually appear somewhere at the edge of the main mass of yolky cytoplasm, and

presumably it was preceded by the formation of something corresponding to a grey crescent, although it is not always possible to recognise this clearly. The position of the blastopore and of the putative grey crescent

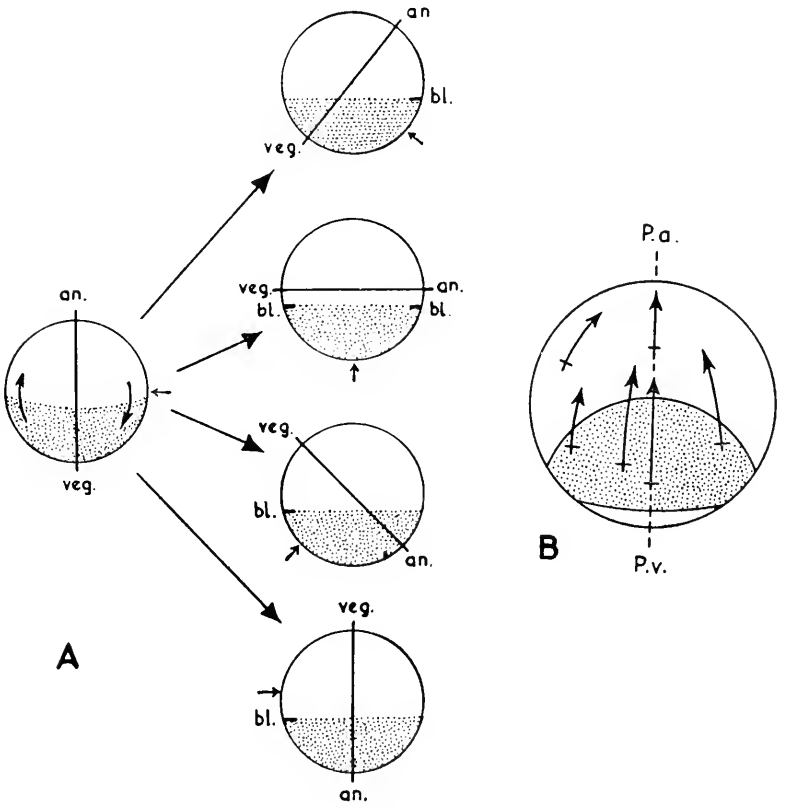


FIGURE 9.3

A. The effects of partial rotation of the uncleaved axolotl egg round an axis perpendicular to the dorso-ventral plane. The small arrow points to the grey crescent and the dots indicate the heavy vegetative oöplasm. Note that the blastopore always appears at the margin of this, in whatever position is nearest to the original grey crescent. (From Pasteels 1951.)
B. The movement of marks on the cortex towards the animal pole (*P.a.*) during the formation of the grey crescent. (From Pasteels 1951, after Ancel and Vintenberger 1948.)

is now, however, not always determined by the direction in which the yolk slid down to the bottom of the egg. It is influenced rather by the position of the original grey crescent which had formed before the egg

was tilted. The blastopore appears at that edge of the yolky mass which is nearest to this position (Fig. 9.3*A*).

Pasteels (see also Dalcq 1950*b*) concludes that the properties of the grey crescent and thus eventually of the blastopore are brought about by the interaction of two factors. The first is a gradient in cytoplasmic constitu-

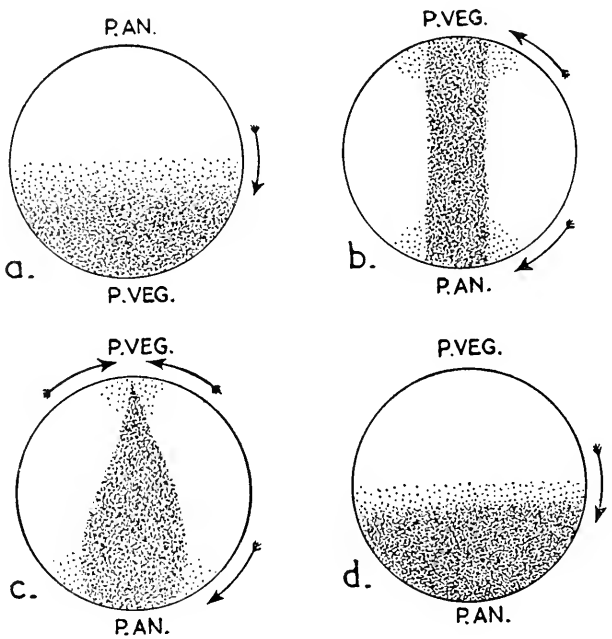


FIGURE 9.4

The relation between the main yolk-mass (dotted) and the anterior-posterior axis of embryos developed following rotation of the frog's egg. The arrows point to the cephalic region of the mesoderm; *a* is the normal situation; *b*, *c* and *d* show inverted eggs in which there is little, considerable or complete redistribution of the yolk to lower pole. Note that the cephalic region of the mesoderm (i.e. the blastopore) always forms near the yolk-mass. (From Pasteels 1951.)

tion which normally runs from the animal pole (rich in cytoplasm and cytoplasmic granules, and poor in yolk) to the vegetative pole (large yolk platelets and little cytoplasm). This gradient determines the cephalo-caudal polarity of the embryo which will develop. It interacts with a cortical field which has a point of highest activity in the future grey crescent region and falls off from that in all directions (Fig. 2.7, p. 42).

It has been necessary to dwell at some length on these early events since

the determination of the plane of bilateral symmetry is, in many ways, of considerably greater importance than anything which happens in the much more striking phenomena of cleavage. Amphibian eggs, having a moderate charge of yolk, undergo cleavages which are definitely, but not exaggeratedly, unequal. The first cleavage plane is vertical, and usually, though not always, coincides with the plane of bilateral symmetry running through the middle of the grey crescent—a coincidence brought about by the fact that the sperm has an influence, again considerable but not quite always effective, on the plane of the first cleavage, just as it has on the plane of the grey crescent. The second cleavage is also vertical, and perpendicular to the first. In many species, the first two dorsal cells are smaller than the two ventral ones, which is an indication that the cleavages are based on a bilateral-symmetrical pattern, which seems to underlie all vertebrate cleavages, although it is often difficult to distinguish.

The third cleavage is horizontal, and the furrow lies above the equator, so that the animal cells are smaller than the vegetative. This is the first indication of the effect of the yolk gradient, whose influence is predominant throughout the remainder of the cleavages. These soon become irregular, and proceed faster in the animal than the vegetative region, so that the difference in volume of the cells becomes progressively more marked. At an early stage—about the fourth or fifth cleavage—a space appears in the centre of the mass of cells, the so-called cleavage cavity or blastocoel. This, of course, lies above the equatorial plane, and, as the unequal cleavages proceed, it not only increases in size, but shifts further and further towards the animal pole.

Cell division continues throughout the whole of embryonic development, but the 'period of cleavage' is considered to end when something else begins to happen. The first definite event which occurs to terminate it is the appearance of the blastopore and the beginning of gastrulation. By this time the egg, which at this stage is called the blastula, has become a hollow ball, with a thin roof of animal cells covering a large cleavage cavity or blastocoel, beneath which lies a floor of large yolky blastomeres.

The most important processes which have been going on under cover of the cleavages have been two. Firstly, the divisions have cut up the egg into cells of a size more attuned to that of the nuclei; and there has been a considerable increase in the total amount of nuclear material, and perhaps a synthesis of DNA, to assist in bringing nucleus and cytoplasm back to their normal relations (but see p. 58). Secondly, there has been a considerable movement of material; if vitally stained marks are made on the vegetative pole of the egg, the dye is gradually carried right into the body of the egg, and eventually reaches the floor of the blastocoel.

The significance of this movement is not yet fully understood (Nicholas 1945). It does not, however, affect the very thin coat which forms the actual surface of the egg. This material, which has special properties of elasticity and toughness, remains on the exterior surface forming the boundary between the egg and the external medium, and seems to play an important part in the biophysics of the morphological changes which lead to the formation of the embryo (p. 439).

The first sign of gastrulation is the appearance of a shallow groove, the blastopore. This lies somewhat below the equator and within the area of light-coloured yolky cells. As was said above, vital staining shows that it appears in the region of the egg derived from the grey crescent, which by this time is no longer recognisable. Before describing the later events, we shall follow the development of the bird embryo up to the corresponding stage, so as to be in a position to compare the gastrulation of the two forms.

(*b*) *The birds*

A discussion of the development of the bird's egg up to the blastula stage will take much less space than was required for the amphibian. This is not because the events are less complex, but because our knowledge of them is less complete. The early stages of avian development remain difficult to explore, partly because the enormous stores of yolk obscure any cytoplasmic differentiation there may be, and partly because the egg at this stage is out of easy reach within the body of its mother, who does not lay it until the cleavage period is finished and the gastrulation begun.

The true ovum of a bird such as the chick does not make up the whole of what we usually call the egg, but only the so-called yolk. This is covered with a well-defined and tough membrane—the vitelline membrane—outside which lies the albumen or 'white' which is again enclosed in a membrane, the whole being finally covered by the shell. All these parts, from the vitelline membrane outwards, are non-living additions to the egg-cell, serving as sources of nourishment or means of protection; they are laid down around the ovum after it has left the ovary, been fertilised, and is on its way down the oviduct. The 'yolk' or true egg-cell is not adequately described by its popular name, since although it obviously contains a very large quantity of yolk, this is by no means the whole or the most important part of it. At one point (which lies in the plane of the smallest section of the ovoid egg) there is a small area of clear cytoplasm containing the nucleus. It is from this that the whole embryo is derived.

The great stores of yolk affect development from the very beginning. Whereas in most eggs, the penetration of one sperm suffices to prevent

the entry of a second, in highly yolky eggs such as birds' this mechanism breaks down, and a number of sperm penetrate. Only one of these completes fertilisation by fusing with the egg nucleus; the remainder form subsidiary nuclei which probably play a part in digesting the yolk in the very early stages of development. In the next events, those of cleavage, the influence of the yolk is even greater. The cleavage furrows start in the clear cytoplasmic region, and never succeed in forcing their way down into the inert mass of yolk. The first two cleavage planes are vertical, but the third, while also starting as a vertical furrow in the flat layer of cytoplasm, soon curves round so as to run horizontally parallel to the surface, and thus cuts underneath the cytoplasm and separates it from the yolk. The cleavage pattern, even in these very early stages, is irregular, and we have little exact knowledge of how the cleavage planes are related to later development.

The cleavages convert the cytoplasmic region into a small compact plate several cells thick. The cells contain fairly large quantities of yolk granules, and at the edges the plate merges into the uncleaved yolk through a zone of increasingly large and more yolky cells. Beneath the mass, however, the yolk begins to liquify, and in sections this region appears as an empty space, the 'subgerminal cavity'. There is considerable debate as to exactly what happens next; and on this turns the question of whether the subgerminal cavity is considered to be equivalent to the blastocoel cavity of the amphibian egg or as a mere local modification of the yolk. The thin plate of cells, lying on the massive yolk, is very easily shrunken and distorted by normal histological methods of fixation and presents great difficulties to the experimentalist; it will not be until ways are found of overcoming these that we shall reach a fully satisfactory interpretation of events. At present, one of the main views (held by Pasteels 1936–7, Peter 1938 and others) is that the subgerminal cavity is not equivalent to the blastocoel, but that the latter soon begins to appear in the form of irregular horizontal splits within the mass of cells; these cavities gradually expand and run together until they form a thin space separating an upper from a lower layer. This space would then be the blastocoel, and the lower layer of cells would correspond with the large yolk-laden cells at the vegetative end of an amphibian blastula. In the bird, these authors hold, the lower layer merely stays where it is during gastrulation and forms the endoderm (Fig 4.1, p. 59).

Others believe that the endoderm, instead of merely splitting off from the upper layer, is derived from it by a more active process which can be regarded as a modified invagination; and they therefore consider the subgerminal cavity, into which the endoderm is pushed, as a true blastocoel.

Different adherents of this view, however, have very different ideas as to the nature of the active process by which the endoderm originates. Perhaps the simplest of these is that of Patterson (1909), who supposed that the posterior edge of the cellular plate becomes folded under and grows forward below the remaining part. This has become one of the commonest accounts given in textbooks, perhaps because of its apparent simplicity; but, unfortunately, the evidence for it is negligible, and all authors who have examined the matter for the last forty years (except Lutz 1953, 1954) have denied it. Another, more plausible, view is that isolated cells are pushed out from the cellular plate and gradually build up a lower layer. Jacobson (1938) believes that endoderm formation begins in this way, but that the process goes on fastest in the posterior region (though not quite at the posterior margin) and eventually attains such an impetus there that the whole plate is bent down into a groove and migrates *en masse* into the endoderm. He describes the formation of a centre of invagination which would really merit the name of a blastopore and closely resembles the structures which, we shall see, are undoubtedly formed at a much later stage when the invagination of mesoderm occurs. Most later authors have been unable to confirm Jacobson's account in full. A final view (Hunt 1937) which must be mentioned is that, however the lower layer of cells is produced at this stage, it eventually does not form the endodermal organs of the embryo, such as the gut, but is at a later stage pushed out to the sides by cells which come out of the upper layer (from the primitive streak, which forms there, see later).

Although it is not possible to decide finally between these possibilities at the present time, the safest view is probably that the greater part of the endoderm is formed in the first way mentioned, by a splitting or delamination of the original cell mass. On this interpretation the blastocoel is not represented by the subgerminal cavity, but by the cleft which separates the upper from the lower layer.

During the period when the lower layer is forming, the mass of cells is also becoming thinner and spreading more widely over the surface of the yolk. From this time on it is usually known as a blastoderm or blastodisc, and its upper and lower layers are frequently—and non-commitally, a useful point in the circumstances—referred to as the epiblast and hypoblast respectively. Moreover, a certain differentiation is appearing in plan. In the more central part, the cells are beginning to have used up their content of yolk granules, so as to become more transparent, while all round the periphery they remain heavily charged and opaque. As the subgerminal cavity becomes more definite below the central region, this differentiation into two concentric zones increases until there is a well-

marked central *area pellucida* surrounded by an *area opaca*. The embryo is formed entirely within the former. The opaque area is concerned mainly with digesting and liquefying the yolk, a process which is carried out chiefly by the underlying cells, which in this region do not separate cleanly from the upper layer, so that the whole zone remains a mass of rather spongy tissue not clearly divided into an epiblast and hypoblast till somewhat later.

By the time the egg is laid, in most birds (such as the chick), the blastoderm has already differentiated into a fairly well-defined *area opaca* and *area pellucida*, and, in the latter, the hypoblast and epiblast are well separated from one another, except perhaps in the anterior region, where the hypoblast may not yet have appeared. Very shortly after this, a thickening appears in the posterior region of the *area pellucida*. This is the beginning of the primitive streak, and the first sign of gastrulation.

2. *Gastrulation: presumptive maps*

At the beginning of gastrulation, the embryos of Amphibia and birds present completely different appearances. The former is a hollow sphere, with a thin roof and a thick floor surrounding a large blastocoel cavity; the centre of gastrulation is indicated by a blastopore, at this stage a small crescent-shaped groove lying within the area of pale yolk-laden cells of the blastocoel floor. The bird embryo is in the form of a blastoderm, a thin sheet of cells floating over a subgerminal cavity filled with liquified yolk; the blastocoel is represented only by a narrow cleft within the sheet, dividing it into an epiblast above and a hypoblast below; and the centre of gastrulation is indicated by the 'primitive streak', a short linear thickening in the posterior region of the *area pellucida*. The end-products of gastrulation in both forms are, however, similar in many essential respects. Both contain three layers of tissue, a mesoderm lying between an outer ectoderm and an inner endoderm. In both an embryonic axis is beginning to develop. An axial strand of ectoderm is thickening and folding up into a tube to become the rudiment of the central nervous system. Immediately beneath this, mesoderm is forming a notochord, a long narrow rod of tissue; while on either side of the notochord the other of mesoderm is condensing into a row of separate more or less cuboidal blocks, the somites. And beneath the mesoderm again, the endoderm is also forming an axial tube, the rudiment of the gut. The movements and foldings by which these two dissimilar starting-points can be brought to these two similar end-products must necessarily differ in many ways; but there is one further point of resemblance which has not yet been pointed out, and which renders the two forms much more easily comparable and understandable.

This lies in the maps of presumptive[1] fate; if we take the centres of gastrulation as our points of reference, these have a similarity which the crude comparison of the blastula and the blastoderm does not exhibit.

Amphibian eggs were the first to which the technique of vital marking was systematically applied to discover the map of presumptive area. The method consists in pressing against some region of the egg a small lump of agar impregnated with a dye which will colour the cells without doing them any great damage. The coloured patch can then be followed through

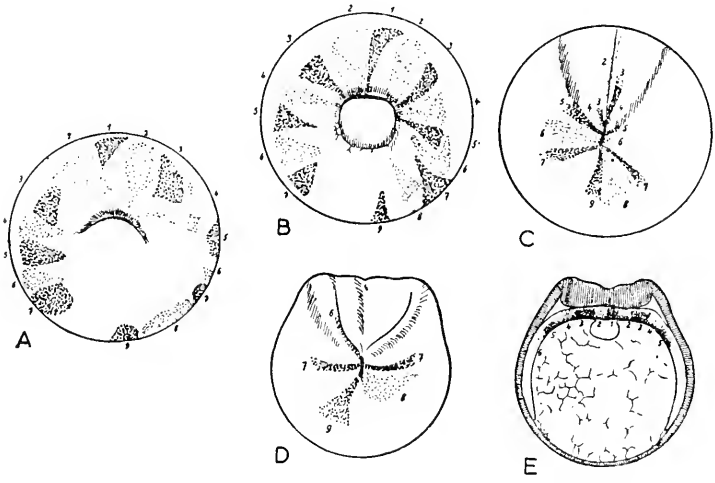


FIGURE 9.5

The movement of vitally stained marks towards the blastopore, and thence into the mesoderm; *e* shows the location of the material in a transverse section of the neural plate stage. (From Vogt 1929.)

its later development, and its fate ascertained (Fig. 9.5). This was done by the German embryologist W. Vogt in 1925, and his results, summarised in 1929, have remained substantially unchanged ever since, although many later authors have modified them in detail (Fig. 9.6).

Vogt showed that the whole lower part of the blastula becomes endoderm and forms the gut and its annexes; the whole upper hemisphere becomes ectoderm, differentiating into the nervous system and the epidermis with its derivatives such as the ears, nasal placodes, etc. In between these two lies a broad belt which forms the mesoderm; on the dorsal side,

[1] The word used by the German authors who originally made such maps was 'präsumptive', and many English authors use 'presumptive' for this: American writers, however, tend to use 'prospective'.

immediately above the blastopore (and thus in the location of the old grey crescent), this develops into notochord; below this region, and further to the side, lie two areas which become the two rows of mesodermal somites, while the remainder of the belt forms the rest of the mesoderm (side-plate, tail-bud, etc.). Thus, if we look at the map from the blastopore outwards, we find first a zone of endoderm within which the young blastopore lies, then a ring of mesoderm, and finally the ectoderm.

The elucidation of the presumptive map in the birds was by no means so simple technically as in Amphibia (summarised: Waddington 1952*a*).

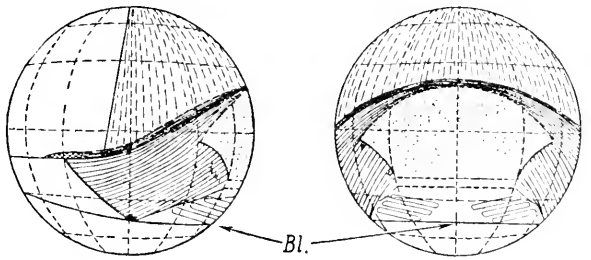


FIGURE 9.6

Map of the presumptive areas of a urodele gastrula. The blastopore *Bl* lies in an area of endoderm (in which the gill slits of the pharynx are indicated). Immediately anterior to it is a wide region of presumptive notochord (dotted), and on either side of that somitic mesoderm (lined), which passes off into ventral mesoderm (unshaded). The animal half of the egg is taken up by presumptive neural plate (dashed), and epidermis (unshaded). On the left, seen from the side, on the right from the dorsal surface. (From Pasteels 1940.)

A beginning was made by Wetzel in 1929, who was able to stain small regions of the blastoderm in the opened egg; Gräper, at about the same time, made speeded-up stereographic cinema films through a window in the shell, from which similar conclusions emerged; and in the next few years, Waddington was able to check many of their suggestions by experiments made on blastoderms removed from the shell and cultivated in tissue culture. Improvements of the methods of vital staining were made by Pasteels (1936–7); and Spratt (1946) has developed a technique of marking parts of the blastoderm with carbon particles, which allows of a more precise labelling, but can only be done on embryos cultivated *in vitro*.

The results of studies by vital staining *in ovo* and by carbon marking *in vitro* have yielded very different maps for the early gastrula, i.e. for the

stage at which the streak is just forming. These are shown in Fig. 9.7. The main difference arises from the fact that Pasteels, in his work with vital stains, finds that during the growth of the streak a considerable movement takes place towards the anterior, while this was not apparent in Spratt's studies. Pasteels' student Malan (1953) has recently examined the matter again, and it has been rather convincingly shown that the absence of this movement in Spratt's material is due to the abnormal conditions of the *in vitro* culture, to which the early stages are particularly susceptible. Thus

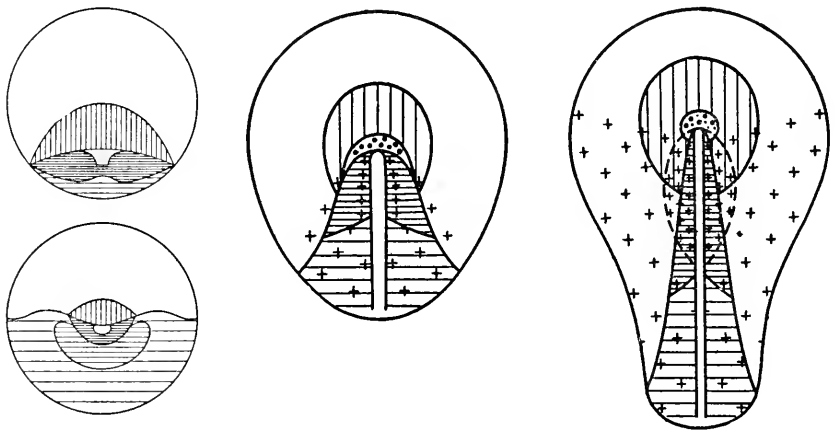


FIGURE 9.7

Maps of presumptive areas in the chick. On the left, just before the streak appears, according to Pasteels, above, and according to Spratt, below (taken from Malan 1953 and Hamilton 1952 respectively). On the right two stages in the formation of the streak (from Waddington 1952*a*). Epidermis, white; neural tissue, vertical lines; notochord, dotted; axial mesoderm, close horizontal lines; lateral mesoderm spaced horizontal lines (the very widely spaced lines in Hamilton's map are extra-embryonic mesoderm); already invaginated mesoderm, crosses.

although Spratt's map has been accepted by most recent American authors (e.g. Hamilton 1952, Patten 1950, Rudnick 1948), Pasteels' earlier one is probably nearer the truth.

In considering this early stage, we are faced, however, with another uncertainty. What is the presumptive fate of the very early primitive streak? Vital staining has shown that in its later stages the streak consists of presumptive mesoderm. But there are great technical difficulties in making critical experiments of this kind on the early stages, and it remains perfectly possible that there is some presumptive endoderm still remaining in the streak when it first forms; this would certainly be so if Jacobson's

account of endoderm formation (see p. 156) were accepted. There is little doubt that in reptiles (p. 234) the endoderm comes from somewhere in this general region of the embryo, and if in birds it originates from a definite part of the surface of the original cell-plate, this must be the place; but, of course, if it arises entirely by delamination from the lower part of mass of cells, there would be no definite location for it on a presumptive map of the surface.

We are on firmer ground when we turn to the other tissues. Vital marks have clearly shown that most, if not all, the primitive streak and the area on each side of it becomes mesoderm, while the area further away takes part in the formation of the ectoderm; the prospective neural ectoderm lies anterior to the mesoderm near the midline.

Comparing the maps of Amphibia and birds, one sees that their general pattern would be similar if one might suppose that the amphibian gastrula has been opened at some point within the area of skin ectoderm, and this hole enlarged until the original map was flattened out to a circle. We should then have an area of endoderm immediately round the blastopore, surrounded by a ring of mesoderm fringing which is an outer ring of ectoderm, with the neural ectoderm concentrated at one end, the anterior. This is exactly the picture we should find in the early primitive streak stage of birds if we suppose that any endoderm originates from the surface. A fuller discussion of the relations between the two groups is given on p. 243.

3. *The gastrulation movements*

(*a*) *Amphibia*

We have now described the state of affairs at the outset of gastrulation and given a sketch of the condition at the end of it. The process of gastrulation consists in the set of movements and foldings which convert the former into the latter. It is clear that, since the bird and amphibian gastrulae differ so considerably, while the early embryos possess roughly similar organs, the two processes must take different courses. These must now be summarised.

Gastrulation in the amphibian is the simpler of the two. It has been followed in great detail by the vital staining technique, but for our purposes it is only necessary to consider the general outlines of events. Before the advent of the staining method, the gastrulation process could only be inferred by comparison of a series of fixed and stained preparations of successive stages. It was natural to try to build up a picture in terms of foldings, delaminations and localised growth; and a series of technical terms, such as 'epiboly', 'emboly', 'invagination', 'involution', etc. were employed in this connection. The newer methods showed that

the process is actually quite different from anything which had been envisaged. The most fundamental type of gastrulation movement is a flowing or streaming one, in which a piece of tissue is carried bodily into a new situation. With the recognition of this fact, the older terms were seen to be not very appropriate, and they have largely disappeared from the literature. An exception may be made for the word 'invagination', which originally meant a massive infolding of a sheet of tissue (such as was described in the infolding of the endoderm in echinoderms (p. 82)), but which is now often used to cover any process by which an originally outer layer is moved into an interior position.

The most obvious change on the surface of the egg itself during the process of gastrulation is the growth, rounding up and final closure of the blastopore. When it first appears, this is a small somewhat pigmented depression lying beneath the equator within the endoderm. Fairly soon it enlarges laterally to form a short groove. This continues to grow longer, and its two ends curve round to a crescent shape, which passes on to a horseshoe and then to a closed oval. Although the blastopore originally lay wholly within the whitish endoderm, by the stage at which it has closed up to an oval, the more pigmented tissue of the animal hemisphere is found to be lying at its edge, so that the outside of the egg is completely dark except for the light spot of yolky cells within the oval blastopore; this is known as the 'yolk-plug' and is a very characteristic and easily recognisable feature. The history of the blastopore is, however, by no means complete; it gradually contracts in area, drawing together above the yolk-plug which is finally covered over and hidden from sight. By the time gastrulation proper is ended, and the first signs of the embryonic axis are appearing, the blastopore has been reduced to a narrow slit which runs in the direction of the embryonic axis.

By following through the history of vital marks, we can see that much more has been happening than the appearance of the blastopore would lead one to expect. Marks made anywhere within the ring of prospective mesoderm are seen to move down towards the edge of the blastopore, to flow over it, and, as can be shown by later dissection, to move away from it again underneath the surface. The lips of the blastopore are, in fact, not fixed positions occupied by a definite tissue, but are mere structural appearances showing where the flow of tissue along the surface turns downwards towards the interior of the egg. This explains how the lip, which was originally made up of the pale endoderm, later becomes lined with the darker material of the animal hemisphere; the pale material has already moved away inside, and the dark material has streamed down to replace it.

The streaming movement towards the blastopore begins when the blastopore is quite small; it always goes on fastest in the region in which the blastopore first appears, and the movement here involves a great stretching and elongation of tissue in the direction of the meridian joining the blastopore to the animal pole. This meridian will become the mid-dorsal line of the embryonic axis when this begins to form; and this part of the blastopore is, therefore, known as its dorsal lip; the ventral lip is the last-formed portion which eventually appears on the diametrically opposed side of the yolk-plug. The material which flows in round the dorsal lip elongates considerably while doing so, and becomes narrower from side to side; this means that material invaginating further laterally

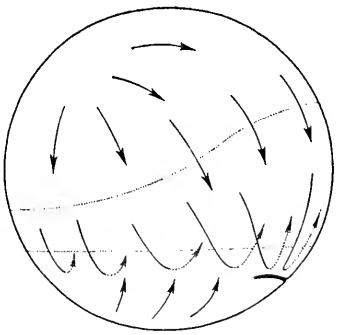


FIGURE 9.8
The gastrulation movements in a urodele, seen from the side.

has to move in towards the midline. The invagination streams, therefore, converge from the sides towards the middle, as shown in Fig. 9.8. Moreover, as the ring of prospective mesoderm moves into the interior of the egg, its place at the surface has to be taken by the prospective ectoderm. We shall see later that the prospective neural ectoderm also elongates along the midline, narrowing as it does so; thus, the whole of the dorsal convergence has to be compensated for by a lateral expansion of the prospective skin. In its crudest outline, therefore, the gastrulation movements of the animal hemisphere of the egg can be summarised as, firstly, a great elongation and narrowing along the dorsal meridian (the elongation being so much that the material flows round the blastopore and finishes up as a double layer), and, secondly, to compensate for this, a lateral expansion, in a plane at right angles to the dorsal one, of the prospective skin at the opposite side of the egg; with, of course, one process

changing smoothly into the other as one goes from the dorsal midline towards the sides.

The process we have described so far would give rise to only two layers, an outer which is the ectoderm and an inner which is the mesoderm. Within this again lies the endoderm, and we have as yet said little as to how it gets there. As a matter of fact, it is the endoderm which starts the whole movement, since the early blastopore lies wholely within it. At this stage, the invagination movement of the endoderm consists in the withdrawal inwards of the main bodies of some of the large endoderm cells, a process which can only be seen in sections (Fig. 20.13, p. 444). A little later the withdrawing endoderm is followed by the first mesoderm flowing round the dorsal lip. As this movement of the mesoderm spreads to more lateral regions, making the lateral lips of the blastopore, the edge of the mesoderm separates from the endoderm to form a free margin such as is pictured in Fig. 9.10; only in the mid-dorsal line is the connection permanently retained at the anterior of the archenteron, and here it has never been easy to decide where to draw the boundary between mesoderm and endoderm. The separation only works round slowly to the ventral side, with the gradual spreading of the blastopore lips. Meanwhile, the region of endoderm which has become free of the mesoderm behaves as though it were sucked in to the interior, folding inwards and at the same time elongating along the dorsal meridian to keep pace with the mesoderm to which its anterior end is still attached. Along the dorsal surface of the endoderm a groove appears, at first shallow, but gradually becoming deeper. This is the primitive gut, or archenteron. As is clear from a section (Fig. 9.9) its walls and floor are made of endoderm, but its roof is at first mesoderm—in fact, the mid-dorsal mesoderm which will later differentiate into the notochord.

Gastrulation is often said to be completed by the time the blastopore is reduced to a small slit. Although this is not actually the case, as can be seen from Fig. 9.10 (see also p. 263), it will be as well to pause in our account of it to notice some of the more obvious changes which begin to occur at this time. When the yolk-plug finally disappears from view, the egg is still spherical in shape, evenly coloured all over with the darker tint characteristic of the ectoderm, and diversified only by the blastopore slit, which is elongated in the meridian of the dorsal axis. Fairly soon after this, the first signs can be seen of the differentiation of the ectoderm into the neural system and the skin. The neural area lies immediately in front of the blastopore, and first appears (for instance in the newt, in which it is well exhibited at this stage) as a pear-shaped or dumbbell-shaped area of somewhat darker colour. The edges of this area soon become elevated as ridges,

while between them the surface is somewhat flattened to make a wide shallow depression. The ridges which mark the boundary of the area are known as the *neural folds*, while the depressed area between them is the *neural plate*. Sections show that the neural plate is thicker than the remainder of the ectoderm (Fig. 20.21, p. 452). As time goes on this thickening increases, the plate simultaneously becoming narrower and the folds higher, until the whole neural area becomes more appropriately referred to as the *neural*

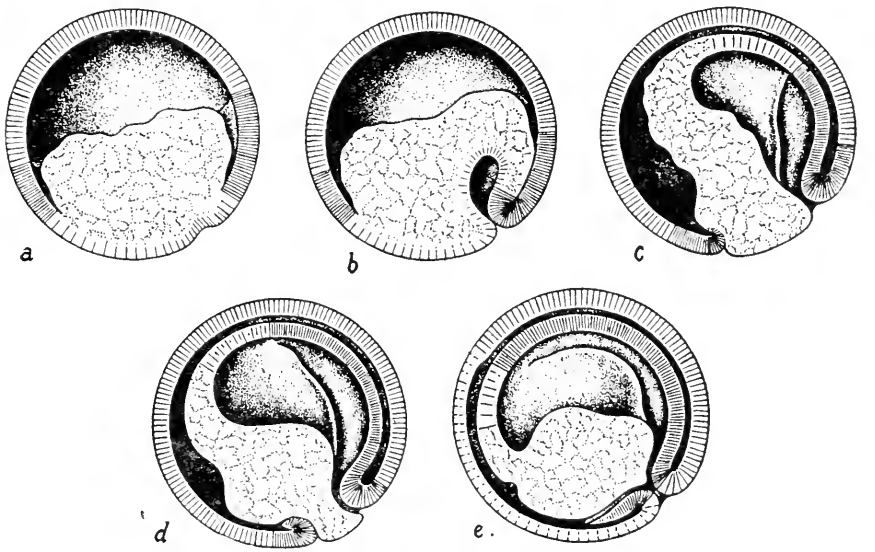


FIGURE 9.9

Semi-diagrammatic drawings of newt gastrulae sectioned through the dorso-ventral plane. The ridge on the wall of the archenteron in *c*, *d* and *e* shows where the endoderm and mesoderm are separating from one another. (From Spemann 1938.)

groove rather than the neural plate. Eventually the neural folds approach so closely that their upper margins touch and fuse with one another; neural material joins on to neural, and skin to skin, so that the neural groove becomes converted into a tube lying beneath an unbroken covering of epidermis. Clearly the tendency of material in this region of the egg to converge towards the mid-dorsal line, which we noticed during the gastrulation movements, has continued even after the stretching in length has become less marked. The same is true of the underlying mesoderm. The central strip which overlies the primitive gut and forms its roof condenses together into a single median strand, the notochord; while the tissue

which lies slightly more laterally also accumulates towards the midline, forming two strips of thickened mesoderm, which soon become segmented transversely, to form two rows of more or less cubical blocks, the somites.

All these changes begin at or near the anterior end, and progress steadily posteriorly; though it should be noticed that the neural groove, being originally wider at its anterior end, does not succeed in closing over so early in its widest parts as it does somewhat further back. At the most posterior end of the embryonic axis, the remains of the blastopore persist

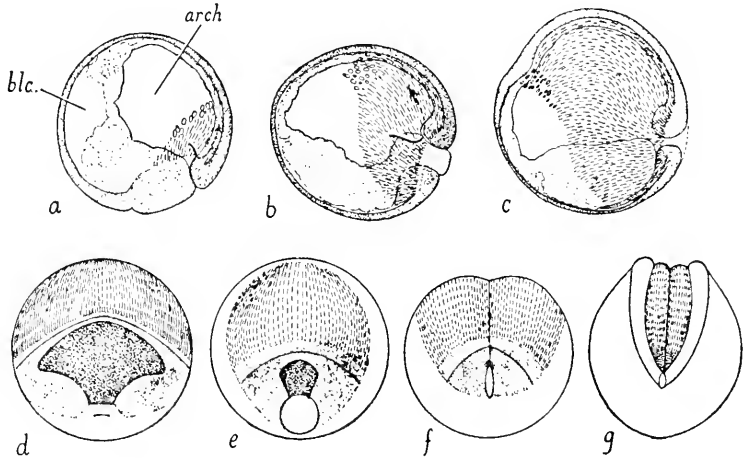


FIGURE 9.10

Sagittal sections at three stages (*a*, *b*, *c*,) of gastrulation in a urodele, to show the expansion of the archenteron and the obliteration of the blastocoel; the extent of the lateral mesoderm is indicated by shading. Below, *d* is a dorsal view on to the blastopore (presumptive notochord closely dotted, presumptive neural system dashed); *e* is a yolk plug stage; *f*, the first appearance of the neural plate; *g*, the neural fold stage. Note that in *f* considerable mesoderm, and even some notochord, is still on the surface, while even in *g* there is a little mesoderm between the neural folds near the remnant of the blastopore. (From Pasteels 1940, after Vogt and Nakamura.)

as a narrow slit; and here, as was hinted above, conditions are still much the same as they were in the more widely open blastopore of the yolk-plug stage, and gastrulation movements still proceed, although on a smaller scale. The notochord, at the dorsal apex of the blastopore, is the first to follow the endoderm completely below the surface, but the invagination of mesoderm round the lateral lips persists for some time longer. Vital staining demonstrates, in fact, that by the first appearance of the neural

plate only the first dozen or so somites have been invaginated, and the mesoderm of the greater part of the trunk, and the whole of the tail, is formed from the small area which still remains on the surface (p. 264). The formation of these organs involves a great increase in length, and the tendency to axial elongation in the midline takes on a new lease of life in this slit-blastopore region. The focus of this elongation is, according to Pasteels, not quite at the blastopore itself, but slightly anterior to it, at the most posterior limit of the invaginated notochord.

(*b*) *Birds*

Although the gastrulation movements in birds are more complex than in Amphibia, they are perhaps easier to visualise and to describe, since they occur not in a sphere, but in a flat circular disc. The main complication which is introduced is a double streaming movement along the midline, forwards at an early stage, and backwards later on. There is moreover another important difference from the Amphibia in connection with the type of growth which is proceeding. In the latter group the total mass of the egg is more or less constant during gastrulation; 'growth' consists in the divison of cells into smaller units, and the conversion into living substance of yolk, already contained in the cells. In the birds, on the other hand, the greater part of the yolk lies outside the tissues, and during gastrulation this is being digested and assimilated, so that growth involves an actual increase in the cellular mass.

We have seen (p. 155) that at the beginning of gastrulation in the chick a lower layer of cells is already present, formed either by delamination or by migration from a posterior region of the blastoderm. This remains more or less *in situ* as the endoderm. It undergoes a general spreading out to cover the whole underside of the blastoderm, which may involve some posterior-to-anterior streaming, but if this occurs at all it is difficult to investigate by vital staining methods, and little is known about it.

The upper layer of the blastoderm consists of the presumptive mesoderm and ectoderm. (For which reason it is sometimes referred to as the mesectoderm, but since this word has been used in other senses, it is better to call it the epiblast.) The formation of mesoderm begins at an early stage. The site of its formation is the primitive streak, the linear thickened area whose appearance in the posterior part of the blastoderm we have taken as the signal for the beginning of gastrulation. At the primitive streak there is no open channel leading from the outside towards the interior, such as one finds at the amphibian blastopore. Nevertheless, vital markings show that tissue which originally lay on the outer surface of the epiblast streams from both sides towards the streak, turns

downwards when it arrives there, and moves away again in a lower layer which lies beneath the surface but above the endoderm, and which is therefore the mesoderm (Fig. 9.11). The first mesoderm to pass through the streak migrates, not only laterally, but also towards the anterior. It does not form part of the mesoderm of the embryonic axis, but finally lies well out to the side. This is in strong contrast to what happens in Amphibia, where the first invaginated mesoderm remains in the dorsal axis. But the difference is merely one of the place at which the invagination of mesoderm begins; in the Amphibia this is the dorsal lip, while in the birds the first part of the primitive streak is in a region corresponding more nearly to the ventral or lateral lips.

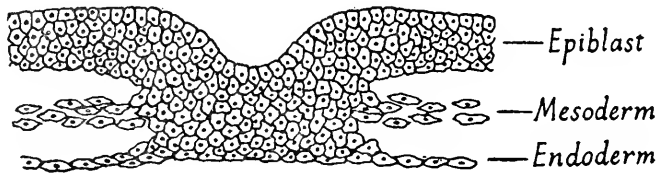


FIGURE 9.11
Semi-diagrammatic section through the primitive streak of the chick, to show the invagination of mesoderm.

Very soon after, or perhaps even simultaneously with, the beginning of this mesoderm-invagination, another movement affects the region of the primitive streak. This is a streaming forwards along the streak towards its anterior tip. As this movement gets under way, the streak lengthens, and pushes out across the *area pellucida*. Whereas when it first appears it occupies only a fifth, or less, of the diameter, it eventually comes to extend more than halfway across the still circular area. While the forward movement is still continuing in the anterior region, the posterior part of the streak starts to push out backwards. When this double movement is at its height, the streak is elongating so fast that it draws out the area pellucida from its original circular shape into an oval or pear-shaped form (Fig. 9.12). The movement bends the regions of the prospective map into long arcs lying on each side of the streak, and since the invagination is continuing all the time, by the close of the forward streaming nearly all the lateral mesoderm has disappeared in the anterior region, leaving the streak bordered by prospective somite material, with a small arc of prospective chorda at the most anterior end (Fig. 9.7, p. 160). The anterior end of the streak becomes somewhat more markedly thickened than the remain-

der, and a slight depression is visible in the centre of it. The structure has been given the name of *Hensen's node*, and a rather exaggerated importance was attached to it in the earlier literature, since some authors held that it alone was the analogue of the amphibian blastopore; actually, we have seen that mesoderm is invaginated throughout the length of the whole streak.

Hensen's node does, however, mark the site of a somewhat special type of invagination. In most of the streak during the phase of forward streaming, the direction of movement of the mesoderm has been in towards

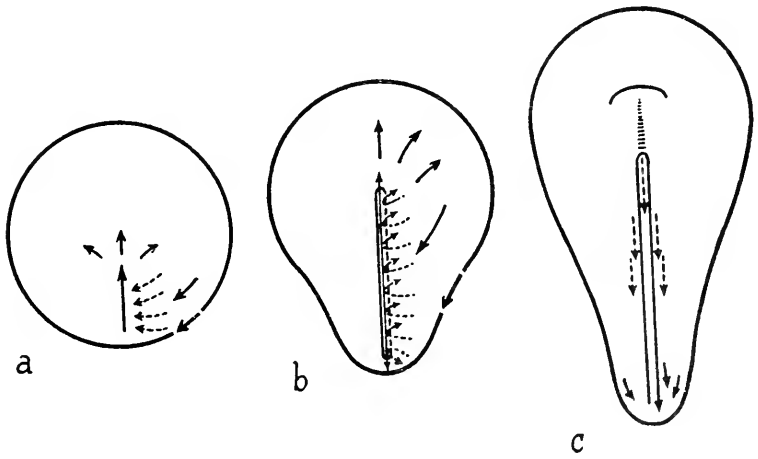


FIGURE 9.12

The tissue movements in the chick blastoderm: *a*, during the elongation of the streak; *b*, in the fully grown streak; *c*, during the regression of the streak. Solid arrows show the movements on the upper surface, dotted ones those at a lower level (i.e. in the mesoderm). (From Pasteels 1940.)

the streak from both sides, and out again in the lower layer towards the sides and somewhat forwards, as indicated in Fig. 9.12. The formation of Hensen's node seems to indicate the beginning of an invagination directed wholly along the midline; tissue, presumptive notochord to be explicit, moves from slightly anterior to the node backwards into it and after sinking to the lower level, passes out again directly forwards, so that a tongue of notochord extends anteriorly to the tip of the streak. This is known as the *head process*.

The formation of the head process brings to an end the forward movement along the embryonic axis, and from then onwards all the streaming movements are directed towards the posterior end. Although, as we saw,

the backward movement started at an earlier stage in the posterior region, it soon acquires relatively greater speed in the neighbourhood of the node, which therefore travels back down the streak, catching up, as it were, the regions posterior to it. The node marks the most anterior point where invagination is still proceeding, and as it moves backward along the streak, the area in front of it is occupied by neural ectoderm in the upper layer and already-invaginated notochord and somites in the lower. Since the node is moving faster than the more posterior parts, the total length of the streak is continually being reduced; but it is a long time before the node finally overtakes the posterior tip of the streak and thus obliterates it altogether.

Before this happens the fundamental organs of the embryonic axis appear at the anterior end much as they did in the Amphibia. The plate of neural ectoderm rolls up into a groove and finally into a tube sunk below the epidermis; the underlying mesoderm separates itself into a median notochord flanked by thickened strips of somite material; and these become transversely segmented to form the paired cubical blocks of the somites themselves. The remnant of the streak which persists in the posterior all this time, where invagination is still proceeding, may be compared with the slit-like blastopore which we saw remains active while the neural groove is forming in the Amphibia; but in the birds the structure is not only relatively larger than in the frog, but continues in being to a stage in which the anterior part of the embryonic axis is much further advanced. The formation of a definite gut from the endoderm will be described later (p. 252).

4. *General properties of gastrulation movements*

We have confined ourselves so far to a straightforward description of the movements which carry the regions of the early gastrula into their final positions. These are the fundamental events by which the future animal acquires its organic form, and the biophysics of the process will be discussed in some detail later (Chapter XX). There are, however, some general points which may be mentioned here.

The forces producing gastrulation are not entirely functions of the egg as a whole, but are inherent in quite small parts of it. This emerges clearly from experiments in which parts of the gastrula are isolated. For instance, if in the stage with a fully formed primitive streak the chick blastoderm is cut transversely into two parts, the expected backward streaming along the axis takes place in both of them. This leads to the protrusion from the anterior part of a 'tail' containing the axial organs (neural tube, notochord and somites), while in the posterior half the medial material withdraws

posteriorly, leaving a gap (Fig. 9.13). The same phenomenon can be seen even in smaller fragments when these are grafted into abnormal situations. Grafts taken from the region of presumptive mesoderm in the newt and placed in some other part of the gastrula, usually succeed in moving below the surface into the mesodermal layer, often forming a small blastopore of their own to do so. Moreover, the direction in which this invagination occurs is more or less definitely implicit in the fragment. Some of the most

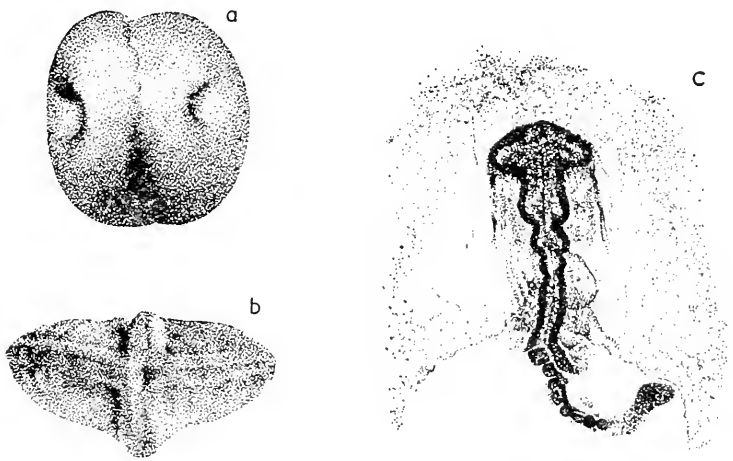


FIGURE 9.13

Tissue movements in parts of gastrulae: *a* shows two vegetative half-gastrulae of the newt placed together so that the blastopores point towards one another. The form which develops (*b*) is a 'duplicitas cruciata'; the two streams of mesoderm from the two blastopores have met head-on, and been forced to spread out to each side, so that each head region is derived half from one egg and half from the other. (From Schleip, after Spemann.) Figure *c* shows the 'tail' developed from the anterior portion of a chick blastoderm transected just behind the node. (After Waddington 1932.)

striking examples of this have been described by Waddington (1941) in the anuran *Discoglossus*, in which the gastrulation movements are very rapid and perhaps for this reason seem to be rather definitely determined in the tissues; if a small part of the dorsal mesoderm of this form is removed and grafted back, reversed in direction, it starts pushing out a tongue of tissue which moves in the opposite direction to that of the main stream of mesoderm which surrounds it.

The inherent movement-tendencies of the parts of the gastrula are, however, at first by no means unalterable, as many experiments have

shown. For instance if two gastrulae are cut in half transversely and the two halves containing the blastopores combined, the two forward-moving streams of axial mesoderm meet one another before they have completed their elongation. As Spemann showed (cf. 1938) they then combine and spread out to the two sides, so that finally double embryonic axes are formed in a cross-shape (a so-called *Duplicitas cruciata*). Again, if a fragment is taken from a region where the movement is not very intense (e.g. the lateral or ventral mesoderm in the newt, or the more posterior parts of the primitive streak in the chick) and is grafted near a region of active movement (such as the dorsal lip of the blastopore or the anterior primitive streak), the inherent tendencies of the graft often appear to be swamped by the more powerful tendencies of its new surroundings. And this does not seem to be merely a question of the graft being physically swept along in the tissue streams of its new location, but the phenomena suggest that the graft is as it were infected with the characteristics of its neighbourhood (Spemann and Geinitz 1927).

This infection of a graft with the dynamic tendencies of its surroundings has an important bearing on the classical problem of 'the specificity of the germ-layers'. The older embryologists, relying entirely on descriptive methods of analysis, tended to reach the conclusion that ectoderm, mesoderm and endoderm were three fundamentally distinct types of tissue from a combination of which the embryo was built up. Soon after methods of experimental attack were discovered in Amphibia, however, Mangold (1925) showed that pieces of prospective ectoderm, if grafted into the mesoderm region in front of the blastopore, became invaginated with their surroundings, and thereafter behaved in every way as mesoderm, and also that ectoderm could be converted to endoderm in a similar way. In birds the demonstration is less complete, but Waddington and Taylor (1937) found that pieces of prospective ectoderm grafted into the primitive streak could become mesoderm, provided they became well enough assimilated to their surroundings for the coherent tissue to break down into single cells which migrate separately into the middle layer. These experiments show that there is no profound and permanent physiological difference between the three layers.

SUGGESTED READING

Vogt 1929 is a classical paper (in German, but the pictures should be studied). The early development of Amphibia is described in many texts (e.g. Nelsen 1953, Spemann 1928). Lehmann 1945, Fankhauser 1948, Dalcq 1950*b*, Pasteels, 1951 add important information on early stages. For the chick, Waddington 1952*a*, Chapter 2, Hamilton 1953, Rudnick 1948.

CHAPTER X

THE EPIGENETICS OF THE EMBRYONIC AXIS

1. *Amphibia*

The account which has so far been given of the development of amphibian and bird embryos has been concerned almost entirely to describe the events which take place; it is time now to see how far we can go in giving a causal explanation of them. Attempts to analyse experimentally the processes which bring about developmental change have of course been made for many years, but it is only in the last three or four decades that we have begun to understand the causal connections involved. This deepening of our understanding may not too unfairly be dated from Spemann's discovery in 1918 of the 'organiser', which will be discussed in some detail below. There were, of course, foreshadowings in earlier years of Spemann's discoveries, as there always are of important scientific advances; but in this case they were so slight, and their importance so little understood even by their authors, that they serve rather to illuminate the magnitude of Spemann's advance than to dim its lustre. It is therefore of great importance to understand exactly what Spemann discovered and the way in which it is significant (Reviews: Spemann 1938, Dalcq 1941, Needham 1942, Lehmann 1945).

Since most eggs are small, the manipulative difficulty of experimenting on them is considerable, and the older experimental embryologists had found themselves restricted almost entirely to the expedient of cutting the egg into fragments, which were then allowed to develop in isolation. The result of such an experiment was normally either that the fragment developed into the fate which would have been in prospect for it if it had remained untouched in the egg, or that it developed into a complete embryo. In the former case, the egg was called a mosaic egg, in the latter a regulation egg; and, if a further step in theoretical analysis were called for, the fragments of the former type might be called 'unipotent' and those of the latter 'totipotent', these words implying that the former had only one potency for development while the latter had all the potencies required to produce a complete organism. Where it was possible to perform experiments on a series of younger and older stages, it was commonly found that while fragments from an early stage might be totipotent, those from a later one had become unipotent.

There grew up a considerable body of discussion of the way in which the

transition took place. For instance the American embryologist Lillie (1929) spoke of a process of 'segregation' or 'differential dichotomy' by which the totipotence of the original egg was sorted out into a set of unipotencies distributed to the appropriate parts of the embryo. But although the words totipotent and unipotent may be quite convenient additions to the embryological vocabulary, it is a mistake to allow their seductive technical flavour to conceal the fact that they suggest no explanation of anything. Molière many years ago made fun of the doctors who thought they could explain the sleep-producing action of a drug by attributing to it a 'soporific quality'; and a 'developmental potency' is a phrase of the same kind. To say that a certain part of an egg has a potency for neural tube formation, for example, means no more than that it has been observed in certain circumstances to become neural tube; and any possibility of providing a causal explanation of the phenomenon lies not in the invocation of potency, but in analysing the conditions under which such a development occurs. Spemann's service was to discover phenomena which allow one to pass beyond such tautological concepts as potency, and take the first step in identifying the causal interactions involved in development. Naturally the revelation of the first step immediately prompts new questions as to the steps beyond; but in science, as in much else, *c'est le premier pas qui compte.*

Spemann's success was partly due to a wise choice of experimental material. The newt's egg is large, and lends itself to the easy performance of grafting and cutting experiments. In the early years of the century Spemann cut the egg in half at various stages from fertilisation onwards, and showed that each half might produce a complete embryo when the operation was made at any stage before gastrulation. During gastrulation, the 'totipotence' of the halves was rapidly reduced, and by the end of it each half gave rise to only a half embryo, whatever the plane in which the cut was made. The crucial problem was therefore to discover what happened during gastrulation to 'restrict the potencies' of the parts.

A clue was present in the fact that although in some experiments both halves of an early stage gave rise to complete embryos, in others one developed completely while the other formed only a mass of cells lacking any sign of the organs of the embryonic axis. We have seen earlier (p. 148) that in fact only those halves containing the grey crescent material develop properly; but in the newt the grey crescent is not clearly to be seen, and the location of the important region was therefore not obvious. Spemann ran it to earth by a series of experiments on the early gastrula. He found that if he separated dorsal and ventral halves, only the dorsal ones developed an embryo; again, if he cut the gastrula in half along the equator,

only the vegetative half developed; thus the crucial region is in the dorsal vegetative quadrant which contains the blastopore. The next step was to graft a fragment from the blastopore region into another location in the egg. It was found to develop, whatever its new position, into part of an embryonic axis (Figs. 10.1, 10.2). Grafts from the presumptive ectoderm,

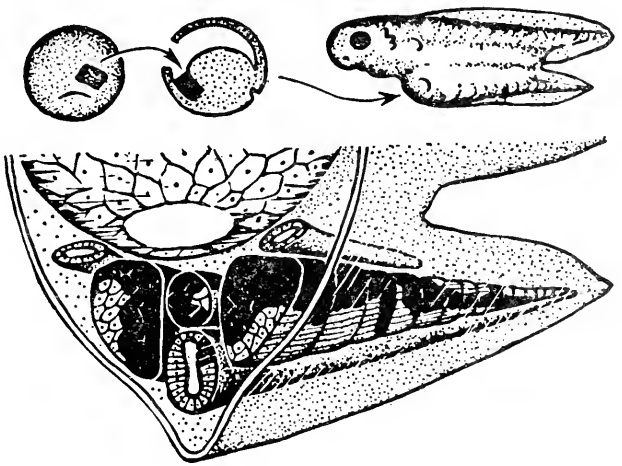


FIGURE 10.1

An organiser graft by the 'Einsteck' technique. A piece of tissue from the neighbourhood of the blastopore of one gastrula is inserted into the blastocoel of a second gastrula. The movements of gastrulation press it against the ventral ectoderm of the host, in which it induces a secondary axis. The diagrammatic view of this (below) shows that the organs may be normal in shape, although formed partly from the graft (black) and partly from host tissues. (From Holtfreter 1951.)

on the other hand, did not behave in any uniform manner, but developed in accordance with their new surroundings.

This not only showed that the blastopore region is the part which is essential for the formation of an embryonic axis, but also suggested that it acts as it were as a focus around which the whole egg is organised. Spemann suggested that when a graft of ectoderm develops similarly to its new surroundings, it is really its new relationship to the blastopore which is determinative. A final proof of this came a few years later, when Spemann and Hilde Mangold (1924) made grafts of the blastopore region of gastrulae of *Triton alpestris* into gastrulae of another species of newt, *T. taeniatus*. The tissues of the two species can be distinguished in stained

and sectioned material and this made it possible to demonstrate conclusively that the grafted tissues had not only themselves developed into parts of axial organs, but had also caused the surrounding host tissue to do so, although its presumptive fate was to become mere epidermis.

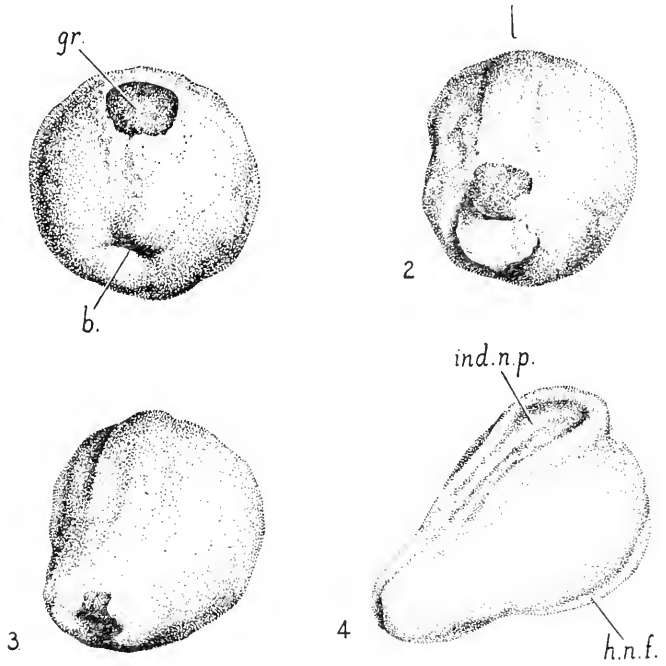


FIGURE 10.2

1. View of vegetative pole of a newt gastrula. The main blastopore is towards the bottom, and a second blastopore region, from another gastrula which was vitally stained with Neutral Red, has been grafted into the ventral side of the vegetative region.
2, 3. Stages in the invagination of the normal and grafted blastopores.
4. The grafted material has become completely invaginated and disappeared below the surface. It has induced a neural plate (*ind. n.p.*). One of the normal neural folds of the host embryo can be seen (*h.n.p.*).
(Original, from a time-lapse cine film.)

Such a reaction was spoken of as an *embryonic induction*, and the region near the blastopore from which inducing grafts can be obtained was called by Spemann the *organisation centre* or *organiser* of the embryo.

The discovery of the organiser gave embryologists for the first time the power to control the direction in which an embryonic tissue develops,

and to do this by means of a mechanism which operates during normal development. Thus a piece of gastrula ectoderm can be made to differentiate into neural tissue by placing it in the near neighbourhood of an organiser. This remains a step of the utmost importance in the history of embryology. It did not, of course, provide a final answer to all the problems of development. Some authors have been so zealous in emphasising this (e.g. Weiss 1935, 1939, 1950*b*) that they have tended to suggest that the whole concept can be dropped from our thinking, which is indeed to throw the baby out with the bath water. What is called for is not a rejection of Spemann's ideas, but a further analysis and clarification of them. The phenomena which he had revealed are certainly complex. Thus 'induction' has two different aspects, evocation and individuation, while an essential role in the whole process is played by the 'competence' of the reacting tissues. We shall have to discuss these concepts further below.

The next few years following the discovery of embryonic induction were naturally spent in a general survey of the organiser's properties. The extent of the organisation centre was examined by inserting small fragments of one gastrula into the blastocoel cavity of another; the invaginating mesoderm of the host presses the graft up against the ectoderm on the ventral side, and if it possesses any inducing power, a new embryonic axis appears there (Fig. 10.4, p. 180). It was found that the organiser is at least as large as the region which will develop into the axial mesoderm, (the notochord and somites); that is, the original grey crescent. But its boundaries are somewhat vague, since the inducing power falls off gradually from the centre of this region. In early stages, some degree of inducing power has been shown to exist even in ventral regions (Dalcq and Huang 1948, Dalcq 1950); and the capacity for induction is quite definite though weak, outside the axial mesoderm in late gastrulae, so that even the lateral parts of the mesoderm can induce when planted into quite young hosts (Waddington 1936*a*). The organiser region is, in fact, a 'field system' in which the peripheral parts are dominated by the centre.

Fragments of early gastrulae which have the power to induce always themselves develop into some mesodermal tissues, although they may also form neural, and even endodermal tissues. This point was very well investigated after Holtfreter worked out a salt solution in which embryonic amphibian tissues would survive and develop, using up their stores of yolk as nutrients. He showed (1938*a*) that isolated pieces of presumptive mesoderm could develop into very many different tissues, although the region from which a given organ was obtained was roughly centred on

that from which it would normally develop (Fig. 10.3). The remainder of the egg had much less inherent capacity. In particular, the presumptive neural plate and the presumptive epidermis were alike in that when

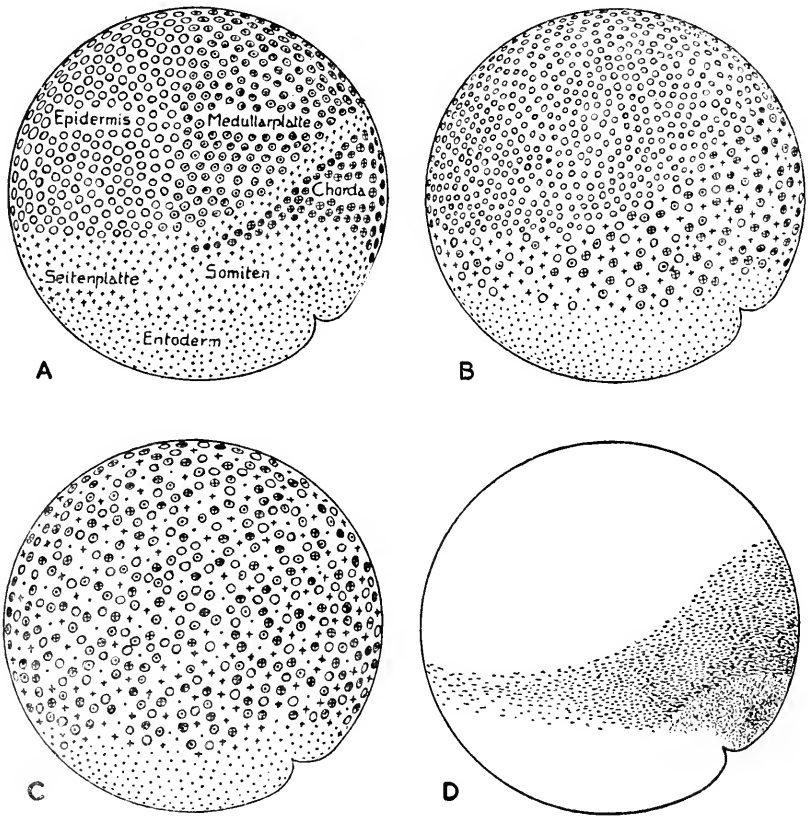


FIGURE 10.3

The urodele gastrula seen from the side; showing *A*, the presumptive fate of the areas (what they will normally develop into); *B*, what they will self-differentiate into when small parts are isolated in saline; *C*, the 'prospective potency', i.e. what they can be induced to develop into; *D*, the region of the primary organisation centre ('head' organiser, dots; 'tail' organiser, dashes). (From Holtfreter.)

isolated they both formed merely a generalised epidermal tissue with no special differentiations. This region of the gastrula must not, however, be considered as completely neutral and characterless. It has one most important property; namely the readiness to react to the organiser stimulus. This property shows no sign of being in existence before the onset of

gastrulation, and is certainly no longer present by the end of it; organiser grafts into old gastrulae (closed yolk-plug stage) no longer produce inductions. During the stage when the gastrula ectoderm can react, it is said to be *competent* (Waddington 1932), and the period when the reaction is possible is the period of competence. These words are also used of other tissues which, at later stages, become competent to react to the organising stimuli, which, as we shall see, are exerted by the various organs as they gradually develop (Chapter XII).

Competence can be thought of as a state of unstable equilibrium; the tissue is poised between two or more alternative paths of development, and may follow one or the other according to the organiser stimuli acting on it. In the case we are now discussing the most obvious alternatives are between the epidermal development path or the neural one. But actually there is a third; the presumptive ectoderm may be converted into mesoderm. This may occur even when the organiser graft is made merely by inserting a fragment into the blastocoel, but it is better shown by grafting a small fragment of presumptive ectoderm into the middle of the presumptive mesoderm just above the blastopore lip; it is then found that the graft becomes invaginated along with the host mesoderm, and takes part in the formation of the host's mesodermal organs (Spemann and Geinitz 1927, Raven 1938). Further, if such a graft, after being allowed to invaginate, is then removed and grafted into still another gastrula, it has now become an organiser itself, and can perform an induction in its new host. We shall return later when discussing the physiology of induction, to this 'infectivity' of the organiser (p. 195).

By the end of gastrulation, the action of the primary organiser is over. The competent tissue has been definitely swung into one or other of its possible types of development; it is now definitely started on the way to becoming either neural tissue, or epidermis, or mesoderm. Within each of these types, a good deal of latitude is still open to it; it is still not finally settled whether it will become brain or spinal column; skin or an ear vesicle or a lens; muscle or mesenchyme or part of the urinary apparatus. But the initial choice of path has been made. One step of development has been, in the usual phrase, 'determined', and if the tissue is allowed to develop at all, it will develop in accordance with that determination.

We have so far discussed the action of the organiser in terms of its effect on *tissue* differentiation, speaking of its results as the formation of neural tissue or epidermis, etc. This is a simplification of what actually happens. The result of an organiser graft is often the production of an induced *organ*, i.e. something in which the tissues are shaped into a more or less definite structure and related to one another as they would be in a part

of a normal embryo. In these organs, parts derived from the graft are often closely intermingled with others induced from the host, both together forming a more or less unitary structure. In such cases, the organiser has done something more than merely throw the host tissues into a certain developmental pathway; it must have specified in detail the particular structures, and parts of structures, which the competent tissues form. Spemann (1931, 1938) followed his original discovery of the organiser phenomenon by showing that different regions of the organisation centre have different properties in this respect (Fig. 10.4). The presumptive anterior regions tend to induce head structures and the presumptive tail regions tend to induce tails. There is thus a regional differentiation within the organisation centre, and the regional properties of a given part can be transmitted to the competent tissues in contact with it. In the early gastrula, the regional structure, although definitely present, is not yet firmly fixed. In the first place, although an isolated piece of the

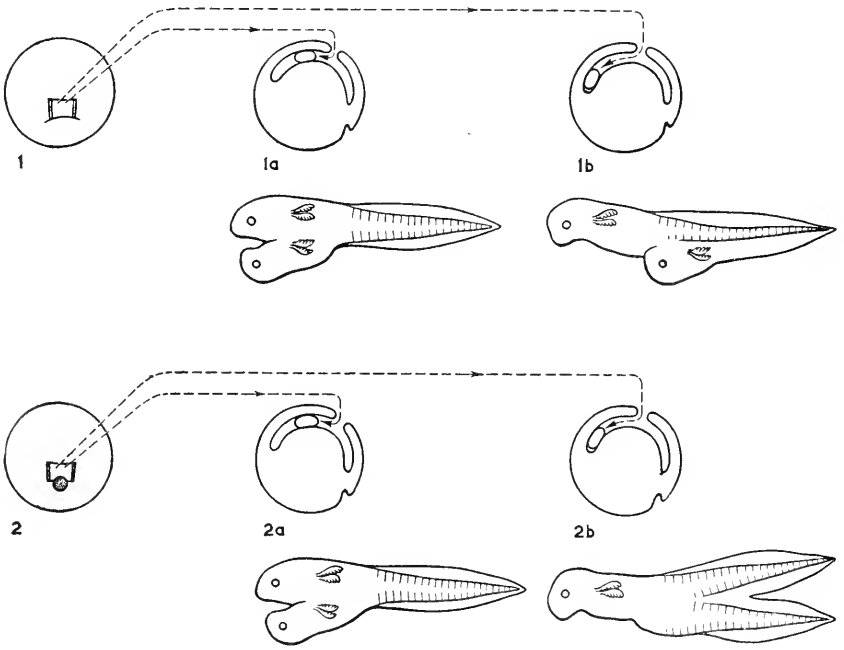


FIGURE 10.4

Head and tail organisers in the newt. *1*, A fragment of tissue (presumptive anterior mesoderm) is taken from near the dorsal lip of an early blastopore and placed in the blastocoel of another embryo so as to arrive at the anterior (*1a*) or the posterior (*1b*) region of the host. In either case it induces a head. *2*, A similar piece taken from a late yolk-plug gastrula may fail to induce a head (*2b*) unless it is near the host's head (*2a*).

organiser has a tendency to form some particular organs of the embryonic mesoderm, it usually develops into, and induces, a larger region of the embryo than it would have done if left in place. Moreover, the different regionalities can influence one another. Tail organisers, grafted into the head region of a host, often induce heads instead of tails. This is not because the competent ectoderm there has an inherent tendency to react by forming a head; it is a consequence of the influence of the nearby host head organiser which tends to force the small grafted fragment to take on the character of the part of the embryo in which it lies. Again, if a small part of the organisation centre is excised and replaced in reversed orientation its surroundings may force it to conform with them, so that a normal embryo results (Abercrombie 1950 in the chick, Waddington and Yao 1950 in Amphibia, see p. 458).

The action of a graft in forming, together with what it succeeds in inducing, a more or less complete organ, and the action of one organiser on the regionality of another are both examples of a tendency by a fragment of organiser to form a whole and complete unit—either a complete organ or a complete embryo. Spemann at first tended to think that this unit-forming tendency was an essential property of the organiser; in fact, the very name 'organisation centre' which he gave his discovery seems to imply something of that kind. However, Waddington and Schmidt (1933) were able to show that this is not the case. The capacity to perform an induction of some kind or another can be dissociated from any tendency to produce the missing parts of a complete unit, or to induce any specific region of the embryo. The two aspects of organiser action can be experimentally separated from one another, and one can have grafts which induce but which cannot truly be said to *organise*. The first clear demonstration of this arose during experiments on the organisers of the chick embryo, and it is now time to turn to a consideration of the epigenetic features of bird development.

2. *Birds*

The study of the epigenetic processes involved in avian development (Review: Waddington 1952*a*) was held up by technical difficulties greater than those offered by the Amphibia. There were not only the usual obstacles of small size, but the embryo is located under a hard shell and viscous albumen, and on top of a fluid 'yolk'. Early experimenters, such as Hoadley, succeeded in cutting the blastoderm in half and following the development of each part; and others, particularly Willier and his students, cut out small fragments of the embryo and got them to develop in isolation by placing them where they could obtain nourishment from

the blood supply of the chorio-allantoic membrane of much older chick embryos. Neither of these methods enabled one to investigate the effect of one part of the embryo on its neighbours, which Spemann had showed to be of fundamental importance in the Amphibia. And, owing to the way the blastoderm is built up of three superposed and closely adherent layers, even the isolated fragments contained a not-well-defined mixture of tissues and were not comparable to the specific gastrula pieces cultivated by Holtfreter in his salt solutions. As a result, very few definite conclusions could be drawn from work which relied on these techniques. Such theories as were suggested were cast in the old terms of 'potencies' and 'embryonic segregation'.

New possibilities were opened up when the technique of tissue culture was adapted to the task of keeping alive the entire blastoderm after removing it from the egg and cleaning it of the adhering albumen and yolk. In such blastoderms, the three germ-layers can be separated from one another, at least in certain regions, and fragments can be grafted into abnormal places where their influence on the surrounding tissues can be studied (Waddington 1932).

We have seen that the morphological changes going on during gastrulation are more complex in the birds than in the Amphibia, and so are the organiser phenomena, probably because the morphological and physiological processes are intimately connected. The first stage in bird gastrulation is the formation of the endoderm, and this is the earliest stage at which an experimental attack has proved possible. In the young blastoderm, in which the primitive streak is just beginning to be indicated, the endoderm may be peeled off, rotated about a vertical axis, and replaced either head to tail, or so that its longitudinal axis makes a right-angle with that of the epiblast (Waddington 1933*a*). It is found that such a rotation has a powerful influence on the elongation of the primitive streak and of the embryo which eventually develops. If the rotation has been through a right-angle, the head end of the embryo is curved round towards the new position in which the head end of the endoderm has been placed. With a complete head to tail rotation the usual result is that the embryo is greatly shortened, and does not extend fully across the *area pellucida*. It is clear that the elongation of the epiblastic part of the embryo tends to proceed in the posterior-to-anterior direction of the endoderm. In some cases of head-to-tail rotation the influence of the endoderm is more far reaching, and a new primitive streak and embryo are induced, running from the posterior part of the endoderm to meet the original embryo head-on in the centre of the *area pellucida*. The endoderm is therefore in some sense an organiser, since it can call forth the develop-

ment of a new embryo. But we do not know how far it can be said to *determine* the developmental fate of the epiblast; probably it only determines that a new primitive streak shall be formed, leaving the later events undecided. As we shall see, a second organiser action has to go on within the streak before the definitive embryo appears. The organising action of the endoderm is perhaps connected with the posterior-anterior streaming which it seems to undergo itself (Fig. 10.5).

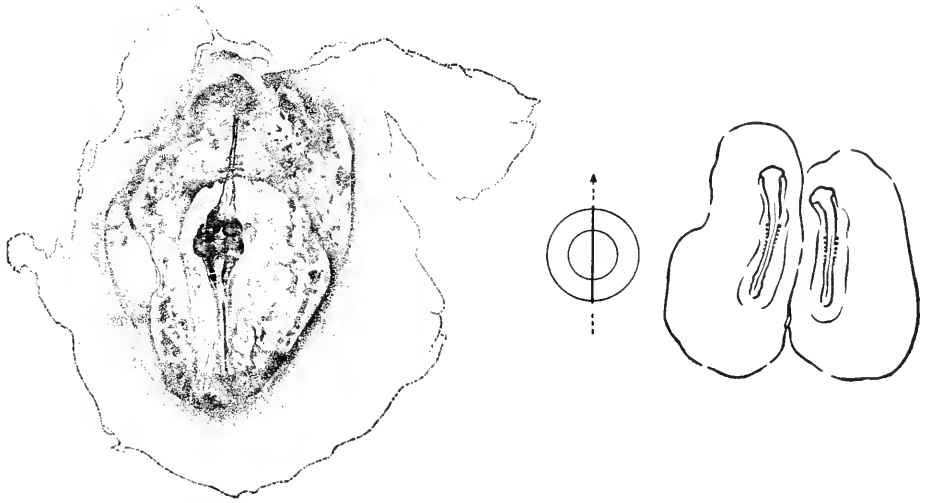


FIGURE 10.5

On the left is a chick embryo, cultured *in vitro*, in which the anterior-posterior axis of the endoderm was reversed in the early streak stage. Two embryos have developed, one (with tail end towards the bottom of the page) in the original direction of the epiblast, the other in the opposite direction; the latter must have been induced by the endoderm. (From Waddington 1933.)

On the right the small diagram shows a blastoderm of the duck, at the time of laying, transected in the dorso-ventral plane; twin embryos develop. After Lutz 1949.)

The early endoderm, in the stages when the primitive streak has not yet appeared, is in a very labile condition. Lutz (1949) has shown that if a duck's blastoderm is cut across at this period, both parts may form a complete embryo, and this is true whatever the orientation of the line of section (Fig. 10.5). The endoderm, then, can regulate very well and its inducing capacity is present, in some degree, throughout the whole of it. If one studies the orientation of the embryos which are formed in this way, it is found that that developed in the posterior part of the blastoderm

always retains its original anterior-posterior axis, whereas the polarity of other regions is more labile. This is an indication that the posterior region is the dominant part of the 'endoderm-field' (Lutz 1952).

There is not much difficulty in making grafts of pieces of blastoderms of the primitive streak stage, once the method of growing the embryo in tissue culture has been adopted. But the procedure differs slightly from that usual in the Amphibia. In the latter, a hole can be cut in the gastrula, and a graft inserted so that it lies flat with the main surface of the egg. If a similar operation is made in the chick, the edges of the wound often curl back, and fail to heal up with the edges of the inserted graft. It is therefore more effective in bird embryos if the epiblast and endoderm are slightly separated, a pocket formed between them, and the graft inserted into it; this corresponds, more or less, to the method of pushing a graft through the roof of the amphibian gastrula into the blastocoel.

The only other important difficulty which arises in the chick is connected with the interpretation of the results. We saw that in the Amphibia the conclusive proof of an inducing action by the grafted organiser was given by making the graft of a different species from the host, so that the two sets of tissues could be distinguished in sections, and convincing evidence produced that parts of the secondary embryonic axis had been formed from host tissues. In birds, it has so far been impossible to find two species whose embryonic tissues can be distinguished with certainty; Waddington and Schmidt (1933) made many grafts between chick and duck embryos and obtained structures which were certainly inductions, but the tissues were similar in appearance, and host and graft could only be recognised in a general way, by the fact that the graft tended to remain a rather separate lump. The final proof of the reality of induction had to be obtained by a different method. This was done by taking two blastoderms, removing the endoderm from each of them, then placing them with the mesoderm faces together, and with the two primitive streaks *not* on top of one another. When the whole of such a combination is grown *in vitro*, as many as four embryonic axes may appear; one from each of the original primitive streaks, and two more, one induced by each streak in the other epiblast against which it lies (Waddington 1932). The neural grooves are still firmly attached to the epiblast from which they arose, and it can be quite definitely seen that the secondary ones have been induced and were not formed from the original streaks. The origins of the mesodermal parts of the embryonic axes is not so certain, and one must guess that they are formed partly from the original streak and partly by induction.

The organising powers of the different parts of the streak can be

investigated by inserting small fragments between epiblast and endoderm, in the way described above (Figs. 10.6, 10.7). As in the Amphibia, the inducing region turns out to be about co-extensive with the presumptive axial mesoderm. In birds, this is a fairly small part of the whole mesoderm, since there is a large proportion of lateral mesoderm destined to migrate

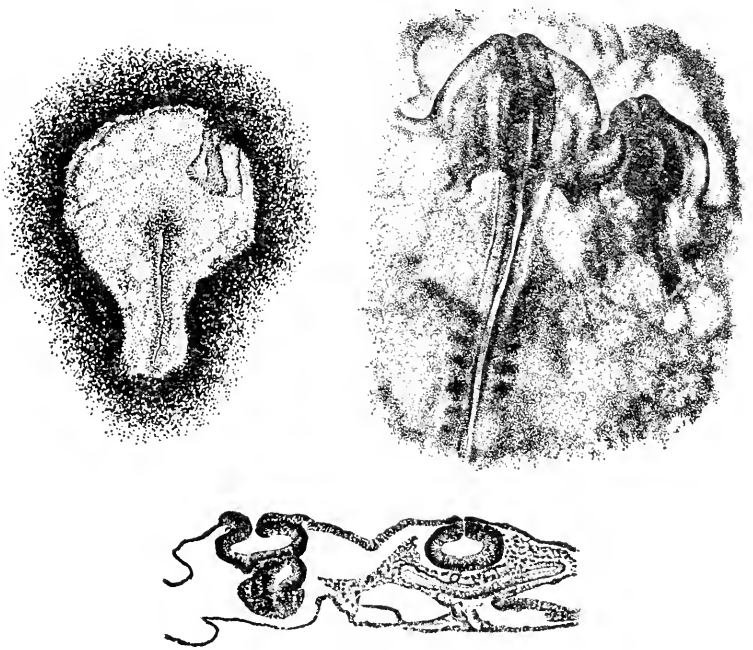


FIGURE 10.6

Above, on the left, a chick blastoderm of the primitive streak stage; at its right anterior a piece of streak from another blastoderm has been grafted between the epiblast and endoderm. On the right, part of a host (duck) blastoderm, with to the right a secondary head region induced by the anterior half of a chick primitive streak. Below, a section showing the induced brain (to the left) underlain by the graft, which has also developed some neural tissue. (After Waddington and Schmidt 1933.)

out to the sides of the blastoderm; this material probably has a wea inducing capacity, and thus, in the fully grown primitive streak stage, grafts from the posterior half of that structure, consisting entirely of the lateral mesoderm, may sometimes succeed in inducing, though they often fail (Abercrombie 1954). There is also some organising capacity in the regions of the blastoderm just lateral to the streak (Abercrombie and Bellairs 1954).

In birds it has not been possible to make any experiments which truly parallel Holtfreter's isolations of gastrula fragments into salt solutions. For one thing, the bird tissues do not contain enough yolk to continue living entirely on their own; they have to be supplied with nutrients, either from the tissue-culture medium or some form of blood supply, and it cannot be assumed that such media are as neutral in effect as is a salt

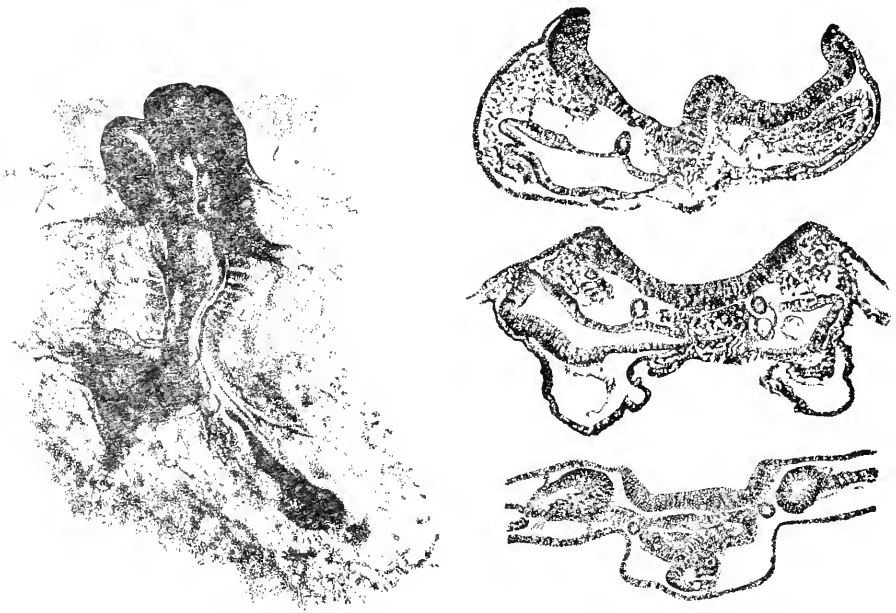


FIGURE 10.7

On the left of the host's axis, an induction has been produced by the posterior half of the same streak used in the embryo shown in Figure 10.6. On the right are three sections, showing how the induced axis (on the right in the figures) has combined with the host axis. (After Waddington and Schmidt 1933.)

solution. Further, the mesoderm and ectoderm adhere closely together in the epiblast, and it is next door to impossible to obtain fragments which contain nothing but presumptive neural tissue. Extensive isolation work has, however, been carried out, chiefly by American embryologists who used fragments of epiblast containing both ectoderm and mesoderm (and often endoderm as well), which were grafted on to the chorio-allantoic membrane of older chick embryos, where they become vascularised and continue their histological, though not their morphological, differentiation. The most important result they have obtained is to show that in the

primitive streak there are rather vaguely delimited areas which exhibit tendencies to produce specific organs, such as heart, eye, liver, etc. (Reviews: Rudnick 1944, 1948). It is probably safe to assume that these rough localisations are, in the first place, characteristics of the mesoderm, and that a tendency to form a definite ectodermal organ such as the eye indicates the localisation of a mesodermal eye inductor rather than of the eye itself (Fig. 10.8). If that is so, the phenomena are essentially similar to the

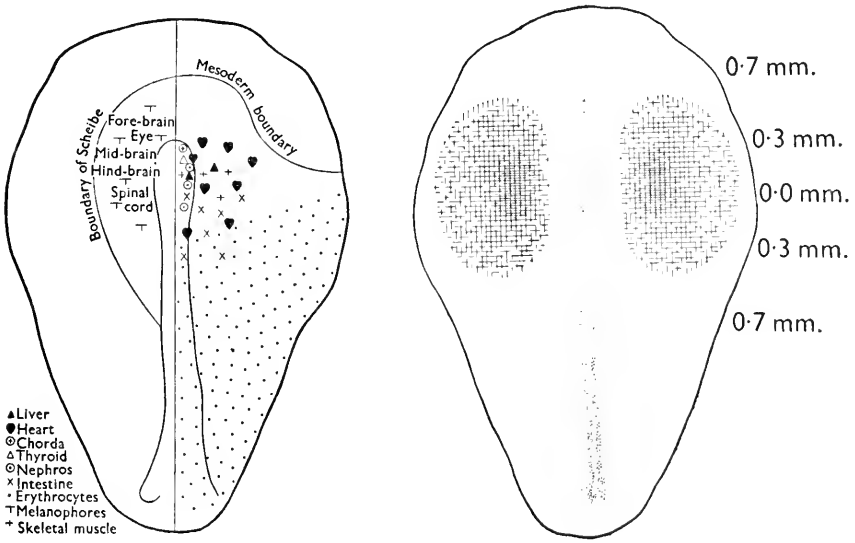


FIGURE 10.8

(*a*) Map showing regions of the definitive primitive streak blastoderm from which various tissues differentiate in chorio-allantoic grafts; ectodermal tissues on left, mesodermal on right. (After Rudnick 1948.)
(*b*) Regions of the head process blastoderm from which heart muscle differentiates in chorio-allantoic grafts; the closeness of the hatching indicates the relative frequence of heart differentiation. (After Rawles 1943.)

vague localisations of different capacities within the amphibian mesoderm described by Holtfreter (p. 177). There is little evidence that the ectoderm in general has any capacity to form neural tissue in the absence of an inducing stimulus from the mesoderm, but there is a possibility that the region which develops into the forebrain may be able to do so even when isolated from mesoderm (cf. Waddington 1952*a*, p. 109).

Other evidence of regionality within the organiser emerges clearly from the results of intra-blastodermal grafts. As in the Amphibia, there is a tendency for grafts of the anterior part of the streak to induce heads,

whereas more posterior parts do not do so unless they are near the head end of the host. This latter point indicates again an interaction between the host and graft, and in the chick this is sometimes very obvious. When a host embryo and an induced one lie closely side by side, they often 'fit' exactly, with each of the organs (head, ears, foregut, heart, etc.) at the same level in each axis, and with the somites exactly lined up (Fig. 10.7). One can find a complete series between entirely separate axes, lying some distance apart, through cases where they are closer and show some degree of fitting, to instances where they have so completely united as to form an almost unitary embryo, whose double origin may be difficult to recognise. However, the experiment described above, in which two epiblasts were placed face to face, shows conclusively that induction is not *necessarily* dependent on a tendency for a part of the organiser to expand itself into a complete organ or embryo; in that experiment, both streaks were quite complete as regards ectoderm and mesoderm, lacking only their endoderm, whereas what they induced was not the missing endoderm, but was the ectodermal neural system (and probably some mesoderm) which they already possessed.

3. *Evocation and individuation*

Facts such as these show that one must take account of two aspects of induction, which will have to be explained by two somewhat separate physiological mechanisms because they can be caused to occur independently of one another. The first of these aspects is the mere calling forth of some sort of an induced differentiation—a process which was originally called 'induction-as-such' and later 'evocation' (Needham, Waddington and Needham 1934). This is independent of any tendency towards the formation of a complete organic unit, and is, for instance, well exemplified in the appearance of the secondary embryos in the two-epiblast experiment.

The second aspect is the formation of an organised structural entity, which may be a whole embryo, or a part of it such as a single organ; for processes of this kind the name 'individuation' was suggested (Waddington and Schmidt 1933). The distinction between these two types of process is quite fundamental for any attempt to formulate theories of development which penetrate deeper than the special embryological level to the underlying biochemical or genetical fundamentals. It is important to realise that the characteristic of evocation is not that the response to it is the production of a small or indefinite rudiment (as suggested by Holtfreter 1951) or one which has no definite polarity or structure (cf. Needham 1942, p. 126). On the contrary an evocation may sometimes cause

the appearance of a well-organised embryo or part of an embryo; but if it does, this embryo must have organised or individuated itself, and its structure will have no connection with any corresponding structure in the evocating material (Waddington 1933*b*). Evocation is an essentially unitary process, in which one single stimulus calls forth some response; whether the response is simple or complex, organised or disorganised, is another matter. Individuation on the other hand is the process by which a structurally organised entity is built up, and is essentially complex, to a degree which corresponds with the number of elements involved in the organisation. It may occur within a piece of isolated tissue (Fig. 10.9C) or a graft which fails to induce (perhaps because its surroundings are too old); or within the rudiment evocated by a graft which does not take part in the individuation (e.g. a dead graft, Fig. 10.9B); or within the graft

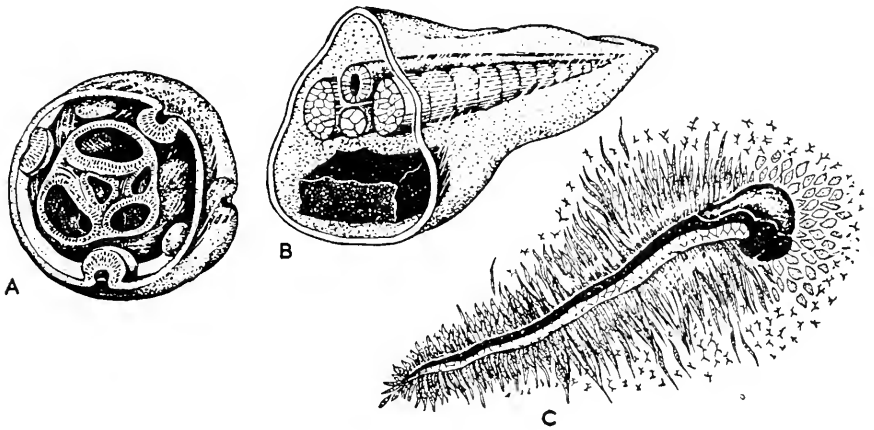


FIGURE 10.9

Phenomena of individuation. *A*, gastrula ectoderm, isolated in an evocating solution, has in part differentiated into chaotic neural vesicles, probably representing parts of the brain, which have induced placodes in the epidermis. *B*, a fragment of adult liver, grafted into an isolated region of gastrula ectoderm, may induce a structure which self-individuates into a well-formed axis. *C*, an isolated fragment of somite mesoderm from the early gastrula tends to develop into a relatively well-organised axis, with central notochord, accompanied by a neural tube which may swell into a brain-like vesicle at one end; there are muscle cells on each side of the axis, and cephalic neural crest cells at the 'anterior'. (From Holtfreter, 1951).

and the induced tissues together (Fig. 10.1, p. 175). Holtfreter gives a clear account of these facts; his criticism (1951) of the concepts of evocation and individuation seems to be mainly about the words to be used rather than about the phenomena themselves.

Whereas evocation may be, and probably is, a straightforward biochemical process, individuation must always involve a biophysical element, since the organisation of an embryonic rudiment is a matter of geometry as much as of the chemical or histological nature of the tissues. Individuation must also usually involve a number of different biochemical interactions, by which the various tissues comprising the organ are brought into being. For instance, in an induction such as that shown in Fig. 10.1 the combined mass of the graft and the induced tissues have developed into neural plate, notochord, somites and nephric mesoderm. The induced neural tissue is immediately in contact with the graft neural tissue, and the same is true of the induced and graft somitic mesoderm, etc. It seems fairly clear that each tissue developing in the graft must have evocated the formation of tissue similar to itself. Mangold (1932) spoke of such phenomena as 'assimilative induction'; more recently some authors (e.g. Medawar 1947) have named them 'infective transformations', and drawn a parallel with the processes of virus infection from cell to cell. We shall discuss later (p. 401) the grounds which exist for such a suggestion.

Individuation certainly also involves other kinds of biochemical induction besides assimilative evocations. For instance, a well-individuated embryonic axis may be induced by mesoderm which itself forms no neural tissue; indeed this is what happens in normal development. Within the mesoderm itself, inductive phenomena, not of an assimilative kind, can be shown to be involved in its individuation. For instance, presumptive lateral mesoderm, if isolated, develops only into mesenchyme and not into somites or nephros, but if some presumptive notochord is put together with it, the lateral mesoderm is caused to differentiate into one or other or both of these tissues (Yamada 1940). It is as though the notochord were at a high point in a gradient of some kind, the lateral mesoderm at a low one, and in combinations some influence diffuses from the notochord and raises part of the lateral mesoderm to the intermediate level corresponding to somites or nephros (Fig. 10.10).

A considerable amount of study has been devoted to the modification which can be made to the mesoderm gradient field by chemical agents. Lehmann (Review: 1945) has shown that lithium ions acting on the gastrula tend to suppress the development of the notochord; the presumptive chorda cells differentiate into a somitic mesoderm instead of into their usual fate; a similar result can be produced by Trypan Blue (Waddington and Perry, 1955). Ranzi (1951) found that thiocyanate has the opposite effect of causing a hypertrophy of the chorda. These facts are exactly parallel to those which have been discovered in echinoderm development:

it is as though the mesoderm of the amphibian gastrula has the same kind of epigenetic organisation as the whole newly fertilised egg of the sea-urchin, the chorda corresponding to the high point of the vegetative gradient and the most ventral mesoderm to the animal pole. Dalcq and Pasteels (1937, 1938, Dalcq 1941) have particularly emphasised this gradient system within the mesoderm, and suggest that it is derived from the two components which were active at the time of formation of the grey crescent, namely, a general gradient in yolk content extending

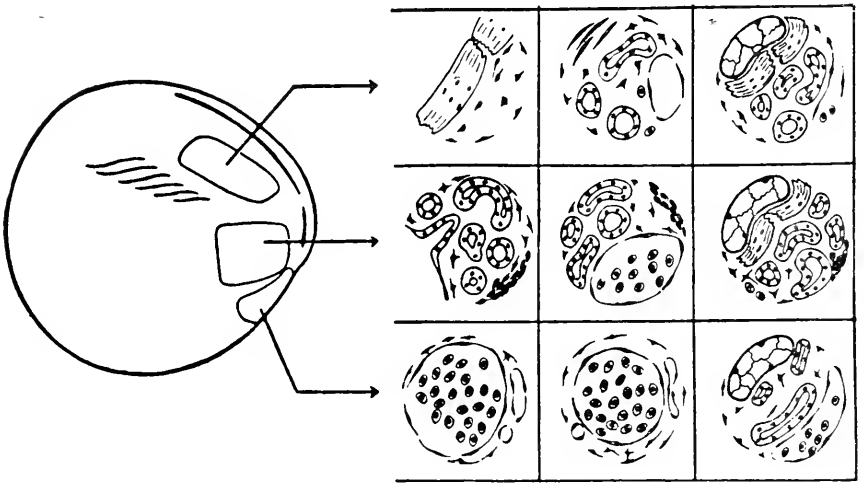


FIGURE 10.10

Differentiation of various regions of the flank mesoderm of the neurula. Column 1 illustrates their presumptive fate; column 2 what they produce when isolated; column 3 what they develop into when combined with a fragment of presumptive notochord. The tissues represented are blood cells, nephric tubules, muscle and notochord. (From Yamada 1940).

through the whole egg from the animal pole to the point of highest concentration at the vegetative pole, and a cortical gradient of unknown nature, located in the outermost layer of cytoplasm and with its highest point at the position where the dorsal lip first appears. The Belgian authors show that one can derive a formal explanation of many phenomena of early development from the interaction of these two postulated gradients; but the concept rather lacks precision when applied to these comparatively late stages of development (see the criticism of Rotmann 1943).

The individuation of an organ or of a whole embryonic axis must be highly complex process in which the various parts of the mass of tissue interact with one another in several different types of induction process. Each individual biochemical interaction can, perhaps, be regarded as an

evocation, somewhat similar to that by which mesoderm calls forth the production of neural tissue. But the whole complex of such interactions, together with the geometrical aspects of the process, clearly form an organised *system* which results in the development of an organ with a recognisable structure. For this reason, individuation can be considered as a typical example of a 'field' phenomenon.

Nearly all developing masses of tissue exhibit some degree of individuation, which may be a self-individuation (i.e. arising autonomously within the mass) or be partly or wholly imposed on it by an inducer. Individuation is least in evidence in fragments of presumptive ectoderm isolated in salt solution; they form quite disordered epidermal tissues. If they produce neural tissue as a result of the action of a structureless evocator (such as a chemical in solution or a fragment of dead tissue), this may also be almost completely without definite form or arrangement, although even in the most disordered cases there is usually a tendency for the neural cells to arrange themselves into tubules and cysts (Fig. 10.9A). Self-individuation may, however, go very much further in such cases, so that definitely recognisable parts of the neural system are formed (e.g. brain, trunk neural tube, etc.); and when the individuation of the earlier stages is fairly well achieved, that of later organs such as the eye, ear, nasal placodes, etc. is often very much better. Isolated fragments of gastrula mesoderm seem always to possess a considerable power of self-individuation, and develop into tissue complexes containing notochord, somites, pronephros etc. with some fairly definite arrangement. It seems likely that the greater tendency to self-individuation in the mesoderm depends on the fact that it develops into several different types of tissue, which can mutually influence one another, whereas the ectoderm tends to form more homogeneous masses. It is noteworthy that the individuation of isolated pieces of mesoderm is better the larger the mass involved, which again suggests that the process depends on interactions between the different parts.

The phenomenon to which the name self-individuation has been applied here has been particularly emphasised by Lehmann (1945). He suggests that when we are dealing with a small lump of tissue which is starting on a course of development, for instance a fragment of presumptive mesoderm or a region of ectoderm which has responded to an inductor, we should always regard this not as a mere conglomeration of cells, but as a '*blastema*'; and this name, which is the Greek word for a bud, is intended to imply a degree of organisation and an interplay of reciprocal influences between its parts.

Rose (1952*a*) has recently suggested that the appearance of different

tissues within a self-individuating region depends primarily on the production of inhibiting agents. He supposes that one region will develop fastest, and will differentiate into some specific tissue. He suggests that while doing so, it produces some substance which can diffuse into the surroundings. This substance is supposed, in the first place, to bring the original differentiation process to an end when a high enough concentration is reached, and in the second to impede the tendency of the neighbouring, more slowly developing, tissue to differentiate in the same direction, and thus to swing it over into some other course. The hypothesis makes a pretty and coherent intellectual scheme, but in this simple form suffers from the grave defect of neglecting the fact that all the evidence suggests that embryonic tissues tend to induce the differentiation of their like, rather than to suppress it. Thus, although Rose claims that extracts of adult frog brain will suppress the formation of neural tissue in the embryo, it is more relevant to normal development that young neural tissue induces further similar neural tissue when placed in contact with gastrula ectoderm (so-called 'homoiogenetic induction'). It is in fact more plausible to suggest that differentiation is usually an 'autocatalytic process', the substance produced by one type of differentiation tending to encourage rather than to inhibit the same type of development. The results which Rose deduces from his postulated set of self-limiting reactions would also follow equally from a system of self-reinforcing reactions combined with competitive interactions (see p. 407). Inhibiting substances of the kind postulated by Rose, may however play a part in regulating the growth of the already differentiated tissues of the young adult.

Rose conceives of the inhibiting substances which he postulates as having an immunological specificity, and operating somewhat in the manner of antibodies. It seems rather probable that developing tissues do influence one another (and themselves) by the agency of substances of an immunological character. The possibility has been discussed extensively by Tyler (1947) and Weiss (1947); the latter author has some evidence that adult organs may differentially stimulate the growth of homologous embryonic ones—just the opposite of what Rose suggests. An adequate body of facts in this field is, however, still to seek. But there are indications that a search for them may be rewarding. One may mention the observation of Ebert (1954) recorded on p. 215.

4. *The physiology of organiser action*

a. Natural and unnatural evocators

This analysis of organiser action into two component parts was soon exemplified in quite another way. During the summer of 1932 both the

German workers on Amphibia and the British on the chick were successful in obtaining inductions by means of grafts which had been killed (Bautzmann, Holtfreter, Spemann and Mangold 1932, Waddington 1933*b*). Now it is fairly obvious that whatever a dead graft may be able to induce, it can hardly produce something which could be regarded as tending to complement the graft and convert it into a complete organic unit, since no dead piece of tissue can possibly form part of a developing embryonic structure. One could therefore consider the possibility that a dead graft might be able to induce something, and even that different regions of a dead organiser might tend to induce different parts of the embryonic axis, but they could certainly not exhibit the whole complexity of the inductive behaviour which is shown, for instance, in the amalgamation of the grafted organiser and what it induces into a complete embryonic axis. In other words, a dead graft might evocate, but it could not individuate. In fact, from the investigation of the capacities of dead organisers, one might hope to arrive at a much more profound analysis of the induction process. Can we perhaps separate evocation again into 'evocation of some generalised sort of neural tissue' and 'evocation of a definite region of the nervous system'? Or is the transmission of regional character always bound up with the completion of a part-structure into an organic whole, and thus necessarily an aspect of individuation? As a matter of fact, we are still not completely sure of the answer (p. 460).

The first important advance beyond the bare fact of evocation by the dead organiser was made by Holtfreter (1934*a*, *b*). He showed that although, when a graft is made from a living egg, only the presumptive axial mesoderm can induce, the properties of the dead material are rather different; the whole presumptive ectoderm and mesoderm, after killing by heat or organic solvents, will call forth the differentiation of new neural tissue. Moreover, many adult tissues, such as liver or kidney, of the most diverse species ranging through the whole animal kingdom, acted as evocators, particularly when killed before being inserted into the blastocoel of a host egg. This appeared at first sight greatly to facilitate the attempts which several groups of workers were making to extract and identify the evocator substance; instead of starting with dead organiser material, which can only be obtained by dissection of the small gastrula, one could start with large masses of liver and test the activity of various fractions. But the different groups of investigators came to quite different conclusions as to which fractions were the most active. Spemann and his collaborators at first identified the evocator with glycogen; Fischer argued that evocation could be brought about by the stimulus of various acids, among which he mentioned the nucleic acids; Needham and Waddington

traced the activity to the fraction of the extract which contained the sterol-like substances, and Waddington demonstrated a high degree of activity in certain synthetic substances of the same nature (Fig. 10.11); while Barth suggested that the evocator substance was cephalin (Reviews: Needham 1942, Waddington 1940*a*, Brachet 1944). Obviously not all of these conclusions could be true; and although some of the claims were mistakes based on the presence of impurities, the situation appeared to be one of complete confusion. It only began to clear up when it was shown that

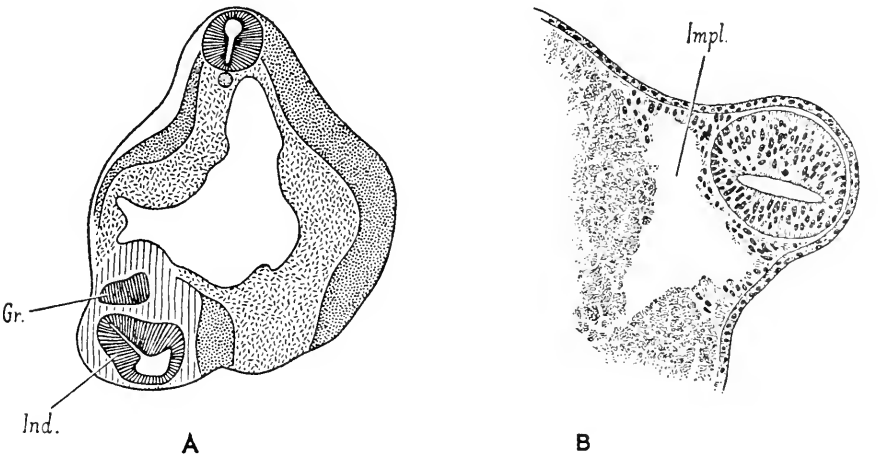


FIGURE 10.11

Diagrammatic section showing a neural tube (*Ind*) induced by a graft (*Gr*) of gastrula ectoderm which had been cultivated for two days in methylene blue. The graft has also formed some neural tissue. *B*, A neural tube induced by an implant (*Impl*) of coagulated albumen containing oestrone. (After Waddington, Needham and Brachet 1936 and Waddington 1938*b*).

evocation could be produced by chemicals which quite certainly are not *the* evocator which occurs in the naturally developing embryo.

The idea of looking for such non-natural evocators arose from another line of thought. In 1927 Spemann and Geinitz had shown that if a piece of ectoderm from the early gastrula is grafted into the region of the organiser and left there for some time, it becomes, as they put it, infected with the ability to induce. Again, various authors have shown that if small fragments of ectoderm are isolated in a situation in which they are bathed by the body fluids (e.g. in the abdominal cavity of a tadpole) they frequently develop into mesodermal tissues such as are normally derived

from the organiser regions (Bautzmann 1929). These facts strongly suggested that the whole of the ectoderm contains all the factors necessary to develop into mesoderm and to induce, and that it is some process occurring at the blastopore region which activates these factors and enables them to take effect.

The well-known axial gradient theory of Child (p. 314) would suggest that the activating process might be something to do with the respiratory metabolism. Waddington, Needham and Brachet (1936) therefore tested the effect on pieces of ectoderm of treatment with dyes known to stimulate respiratory processes. The dye used was methylene blue. It was found, as had been surmised, that if small pieces of ectoderm were isolated in a salt solution containing this substance they sometimes developed into neural tissue, and, if implanted into a young gastrula, were able to induce a neuralisation of the competent ectoderm against which they lay (Fig. 10.11). The dye was, then, acting as an evocator. It would however be ridiculous to suppose that the normal amphibian egg contains methylene blue. It was therefore proved that evocation can be performed by substances other than the substance, whatever it is, which produces that reaction in normal development. This finding put the whole investigation of the nature of inducing action on to a new basis, since, if any substance is grafted into a gastrula and is found to cause neuralisation, that fact cannot be taken as evidence that the substance is the same as, or even necessarily nearly related to, the natural evocator.

There are several mechanisms by which such unnatural evocators might be supposed to operate. In the first place, we have just seen that Holtfreter (1934*a*) had shown that if non-inducive tissue of the gastrula is killed it thereby acquires the power of induction. There is therefore the possibility that if, in a piece of ectoderm, a certain number of cells were killed they might release sufficient evocating substance to induce the remainder to develop into neural tissue. There is little doubt that processes of this kind can occur. For instance Okada (1938) has brought about inductions by mechanical irritants such as silicious earth and Holtfreter (1945) has done the same thing by killing a certain number of cells with a glass needle. It seems certain however that this is not the only way in which unnatural evocators act. There is no sign of excessive mortality of the cells at the concentration of methylene blue utilised by Waddington, Needham and Brachet; and this has also been pointed out by Pasteels (1951) who confirmed the activity of this substance. Moreover Waddington (1940*a*) found that the very actively evocating steroid substances tend to stimulate the rate of growth in ectoderm submitted to them rather than to operate as depressants or cytolytic agents. Holtfreter (cf. 1945) held

for some time to the view that the unnatural evocators acted mainly through the cytolytic mechanism, but he eventually (1948*b*) found himself driven to speak of a 'sub-lethal cytolysis', a somewhat question-begging term which, in effect, admits that the unnatural evocators alter the metabolism of the cells on which they act without actually leading to cell death.

One may take it then that the active substances cause some change in the cell metabolism in the competent ectoderm. We have therefore to conclude that all the factors necessary for development into nervous tissue (and also into organiser derivatives such as chorda, somites, etc.) are already present in the gastrula ectoderm, but require activation before they can be effective.

b. The specificity of the evocator

A certain amount of discussion has gone on in the literature as to whether the unnatural evocators can be considered as 'specific' or 'unspecific' stimuli. It is rarely that very definite meanings have been attached to these two terms. Perhaps the situation should be envisaged as follows. Let us suppose that, in normal development, a substance, *a*, diffuses from the archenteron roof into the competent ectoderm and sets going a process, *b*, which in turn gives rise to process *c* and *d* and so on, until neural tissue is fully differentiated. The hypothesis originally put forward by Waddington, Needham and Brachet, and still supported on the whole by Waddington (1940*a*), Needham (1942), was that substance *a* already exists within the ectoderm but inactivated in some way, perhaps by being combined with some other substance, *x*, to form a complex *ax*. Then the abnormal evocator was envisaged as causing the breakdown of *ax* and the liberation of the active *a*. According to this scheme the sequence of processes *b*, *c*, *d*, etc. can only be set in motion by one specific substance, namely *a*. Alternatively we might suppose that the various unnatural evocators can act immediately on process *b*, setting it in motion and thus leading to *c*, *d* and so on. This would be called an unspecific stimulus because *b* is supposed to react, not only to *a*, but to all the other possible unnatural evocators. It must not be forgotten however that even in the first case, although *b* requires a specific stimulus to set it off, the inactive complex *ax* is supposed to react unspecifically to any of the abnormal evocating substances. Thus a critical point as regards these two alternatives is whether, when abnormal evocators act on competent ectoderm, a substance appears which is the same as that which normally diffuses from the mesoderm into the ectoderm in normal development. Since, as we shall see, this substance cannot yet be identified, the question

is at present unanswerable. The problem of whether the evocator stimulus is specific or not in this sense is therefore, although an interesting one, not profitable to discuss further at the present moment (Fig. 10.12).

There is however rather a different sense in which the terms specific and unspecific can be used. If the evocating stimulus is wholly unspecific, then when used on one and the same type of tissue it can only produce one result. Now this is not the case. We know that in normal development ectoderm differentiates into different parts of the nervous system (e.g.

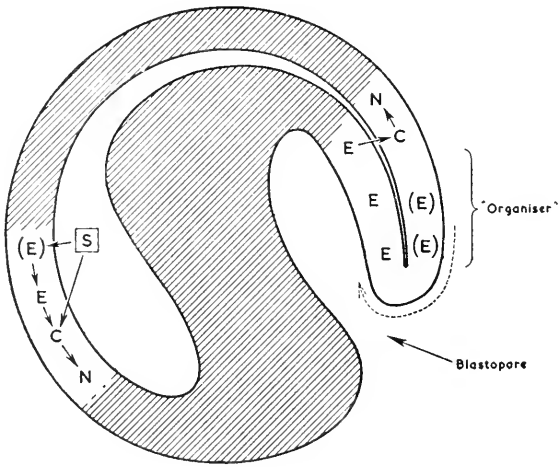


FIGURE 10.12

The activation of the evocator. At the blastopore, something occurs which converts the inactive tissue into an active evocator (change of (*E*) to *E*); this can then act on the competent tissue *C* to produce neural differentiation *N*. If a foreign substance *S* is placed in the blastocoel, and produces an induction, it might do so either (i) by acting on *C* (direct evocation), or (ii) by acting on (*E*) and converting it into *E* (indirect evocation).

brain, spinal cord, etc.). Further, when it is acted upon in a particular way by the living organiser (e.g. by being grafted into the centre of presumptive mesoderm) presumptive gastrula ectoderm can be caused to develop into notochord, somites, etc. Quite a number of evocators or evocating conditions have now been investigated and it has become clear that they do not all result in the same one out of this gamut of possibilities; they are therefore not unspecific in this sense.

The methylene blue inductions did not live long enough for their detailed characteristics to become clear. Some years later, however,

Barth (1941) showed that the gastrula ectoderm of the axolotl (*A. punctatum*) developed sometimes into neural tissue when isolated in, as he thought, completely neutral salt solutions. This was a startling claim because until that time it had always been held that ectoderm could only become neural if definitely induced to do so, and Barth's result seemed to be putting this in doubt. The matter was reinvestigated by Holtfreter (1945) who found that the truth of the matter is that *A. punctatum* ectoderm is particularly sensitive to abnormal external conditions and reacted by neuralisation to salt solutions which have no particular effect on the ectoderm of other amphibian species. If however the salt solution is made to depart considerably from the optimum (by a considerable raising or lowering of pH or by lack of calcium), it can evocate neuralisation even in the more resistant ectoderm of other species. Now in such experiments the evocated neural tissue may develop by self-individuation into a fairly well-defined nervous organ. This organ always belongs to the anterior end of the brain (the forebrain or archencephalon). In considerable contrast to this is the result of another type of unnatural evocating condition. Pasteels (summarised: 1953) has shown that fairly mild centrifugation of early gastrula ectoderm will often cause it to develop into neural tissue and also into notochord, somites and other mesodermal derivatives. The ease with which the action is produced varies in different amphibian species. The point to note is that once again definite organs may be produced and, in this case, they never belong to the archencephalon but always to the posterior end of the brain (deuterencephalon) or spinal column. Finally, Yamada (1950) has found that gastrula ectoderm may be caused to develop into mesoderm by treatment with ammonia.

These differences in the results of evocation make rather unplausible the suggestion which has sometimes been put forward (e.g. by Barth) that the evocator reaction is like that of the artificial parthenogenesis. Recent results have only added confirmation to the conclusion reached by Waddington (1940*a*) that, if the competence of the gastrula ectoderm is set on so fine a hair-trigger that any of a number of stimuli are sufficient to touch it off, we have to admit that the ectoderm can, unlike the egg, shoot in more than one direction. We must in fact be dealing with an orderly system of alternative processes in which the end-result is related in a rather direct way to the nature of the initiating cause. If this were not so, we could expect to get very little profit from an analysis of the evocators, and would have to confine our attention solely to what we can discover as to the processes going on in a reacting ectoderm. As it is we can find important clues to further understanding not only in the ectoderm

itself but also in the nature of the evocators and in the conditions which convert gastrula ectoderm into organiser.

c. The metabolism of the organiser

It will be convenient next to discuss the latter problem (Reviews: Brachet 1944, Needham 1942, Boell 1948). Child's theory of axial gradients would suggest that the blastopore region, which is undoubtedly of extreme biological activity, should have a higher rate of respiratory metabolism than the rest of the gastrula; and we have seen that methylene blue, a well-known stimulant to respiratory processes, can cause the release of evocating power in presumptive ectoderm. There are, obviously, considerable technical difficulties in measuring directly the respiration of pieces of tissue as small as the blastopore region, and the first attempts to compare its activity with that of other parts of the embryo led to rather contradictory results. There is no doubt that the consumption of oxygen rises fairly rapidly in the dorsal region of the neurula, when the tissues of the embryonic axis are differentiating. If one wants to assess the metabolism of the organisation centre at the time it exerts its main inductive effect, it is necessary to have an instrument which is sensitive enough to give an accurate reading of the oxygen uptake within the short period of gastrulation. It was not until Needham adapted the Cartesian diver technique of Linderström-Lang that this requirement was fully met.

Using this instrument Needham and his co-workers (see Boell, Needham and others, 1939) found that in most series of experiments there was no appreciable difference between the rate of oxygen uptake by the blastopore region and by a piece of tissue from a similar position on the ventral side of the egg. This confirmed the conclusion of earlier work with a less-sensitive instrument by Waddington, Needham and Brachet (1936), but there were other experiments, by Brachet (1936), Brachet and Shapiro (1937), Fischer and Hartwig (1938) which seemed to show a higher activity in the blastopore region. The situation was cleared up by Boell (1942) and Barth (1942), who measured the respiratory rate of a series of isolated fragments from all different parts of the gastrula. They found that there is indeed a gradient in respiration. Its high point, however, is not at the blastopore but at the animal pole, and it falls off from there to reach its lowest in the yolky endoderm. The actual figures given by Boell are: Q'_{O_2} (= mμl. O_2 per μg nitrogen per hour) 4·9 for presumptive neural plate near animal pole, 2·1 for dorsal lip, 2·8 for posterior presumptive ectoderm, 1·3 for endoderm. There is, of course, more yolk in the endoderm cells than in ectoderm, and since this is a relatively inert material

which would not be expected to consume oxygen, a better comparison would be obtained on a basis of yolk-free cytoplasm rather than of the nitrogen content of the whole cell. An approximation to this can be reached by crushing and centrifuging the various regions of the gastrula and estimating the percentage of volume occupied by yolk. Applying this correction, the Q'_{O_2} of the active cytoplasm for the four regions is 7·3, 4·8, 5·2 and 3·8 (Boell 1948) (Figs. 10.13, 10.14). Boell suggested that one should probably also make a further correction for non-yolky but non-respiring cytoplasm; and if this is done, the gradients vanish. Sze

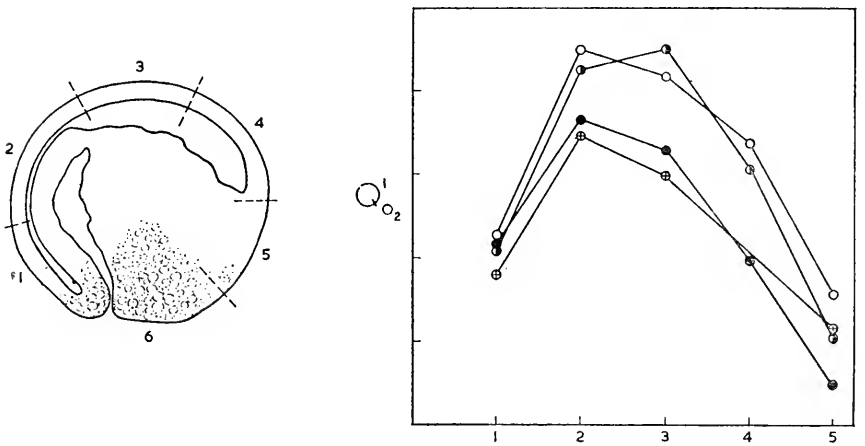


FIGURE 10.13

On the left, a section of an axolotl gastrula, divided into regions, whose relative respiratory rates in four different experiments are shown at the right. (From Boell 1948.)

(1953*a*) also finds that in the frog egg, although there are animal-vegetative and dorso-ventral gradients of respiratory activity reckoned on a dry weight basis, all regions respire at the same rate when compared in terms of their content of extractable (=active?) protoplasm. Flickinger (1954) finds that the rates of incorporation of radioactive CO_2 into the different regions are related in a similar way. It seems then that there is nothing special about the rate of oxygen uptake of the blastopore region; it falls simply into its place in a gradient between the animal and vegetative poles.

The rate of oxygen uptake is, however, by no means the only factor involved in respiratory metabolism. Brachet (1936) found that there is a higher output of CO_2 from the blastopore region than from comparable

ventral regions. Boell and Needham (1939) used the Cartesian diver to obtain more accurate measurements of the respiratory quotient (oxygen uptake divided by CO_2 output). They found that in the blastula roof the respiratory quotient is about 0·75. By the mid-gastrula, this has risen to 1 in the blastopore region, but is still only about 0·8 on the ventral side, where it rises much more slowly and does not surpass about 0·9 by the end of gastrulation.

A respiratory quotient of unity is often taken to indicate that the respiratory metabolism is involving the breakdown of carbohydrate

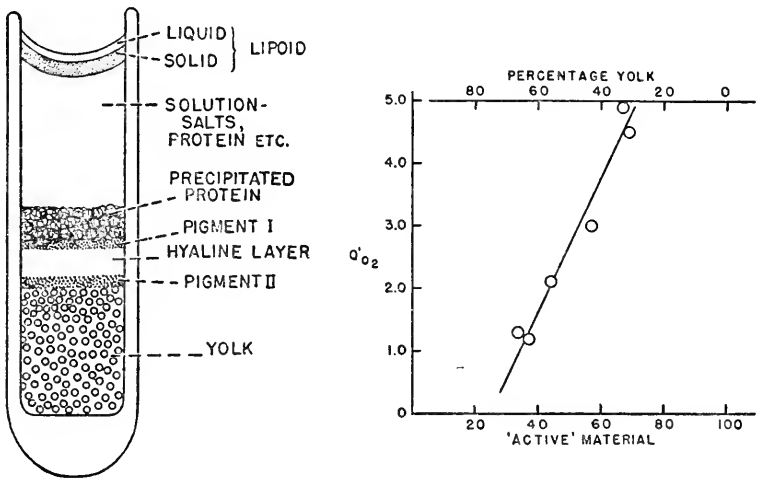


FIGURE 10.14

On the left, a diagram of the stratification of gastrula tissues following high speed centrifugation. On the right, the respiratory rates of the various regions of the gastrula plotted against their content of 'active' material (i.e. everything except yolk). (From Boell 1948.)

rather than of fat or protein. There is independent evidence that this is in fact the case in the amphibian organiser region. Woerdemann (1933) claimed, on the evidence of histochemical investigations, that glycogen disappeared from the presumptive mesoderm cells as they invaginate through the blastopore. Although Pasteels disputed the validity of his methods, Heatley and Lindahl (1937) demonstrated by microchemical analysis that there is a rapid fall in the glycogen content of the invaginating cells, although it does not disappear entirely.

The Cartesian diver studies also showed that the rate of anaerobic glycolysis is about three times as high in the blastopore region as it is on

the ventral side (measured in terms of nitrogen content of the whole cell). There is, however, very little glycolysis in any part of the embryo when oxygen is available.

There seems good evidence, therefore, that the organiser region is characterised by a particularly active breakdown of glycogen, although it does not absorb more oxygen than other comparable parts of the embryo. It is tempting to suppose that this carbohydrate metabolism may be connected with the release of the evocator within the invaginating mesoderm; but that conclusion is not the only one which might be put forward. It is, perhaps, even more probable that the breakdown of glycogen provides in the main the energy which must be utilised in the performance of the movements of invagination (cf. Jaeger 1945). The direct oxidation of glycogen is not essential for gastrulation, since many species, particularly of toads, can gastrulate under anaerobic conditions, or in concentrations of cyanide which inhibit 90 per cent of the normal oxygen uptake; some other species (e.g. the frog) are more sensitive and unable to gastrulate under such conditions. (Cleavage is always relatively independent of oxygen in the amphibia). However, Brachet (cf. 1944) found that iodo-acetate, which inhibits the breakdown of glycogen both aerobically and anaerobically, brings gastrulation to a standstill without impairing the inductive power of the organiser. Although the arrest of movement does not occur till the yolk-plug stage, this evidence rather supports the suggestion that the glycogen is being used to provide energy for invagination rather than in direct connection with the evocator.

Barth (see Barth and Barth 1951) is studying the mechanism by which the embryo utilises the energy derived from glycogen, and also that made available when the yolk is digested at a later stage. He finds that high-energy phosphate bonds, such as those in adenosine triphosphate, are involved, and suggests that the chemical system has some similarity with that characteristic of muscle (see also Dainty *et al.* 1944 and Fujii *et al.* 1951).

At the beginning of gastrulation, certain other alterations take place in the metabolism of the egg, and again certain of them appear to go fastest in the blastospore region. A fact which emerges from cytological observation is that in the gastrula the nuclei become suddenly smaller and stain more deeply with DNA dyes (such as the Feulgen reagent) while nucleoli containing RNA make their appearance. These changes presumably indicate an increased synthesis of nuclear RNA and possibly of DNA too (Brachet 1952*a*). They occur more or less simultaneously throughout the animal cells, but more slowly in the endoderm, where the nuclei remain large. Sirlin and Waddington (1954) found, in autoradiographs of

embryos treated with radioactive amino-acids, that these are incorporated more rapidly into the nuclei than into the cytoplasm at the early gastrula stage, and that this incorporation, which is probably a sign of synthetic processes, begins first in the dorsal lip region (Fig. 10.15).

A little later, when gastrulation is under way, there seems to be an increase in the cytoplasmic RNA. This can be demonstrated by measuring

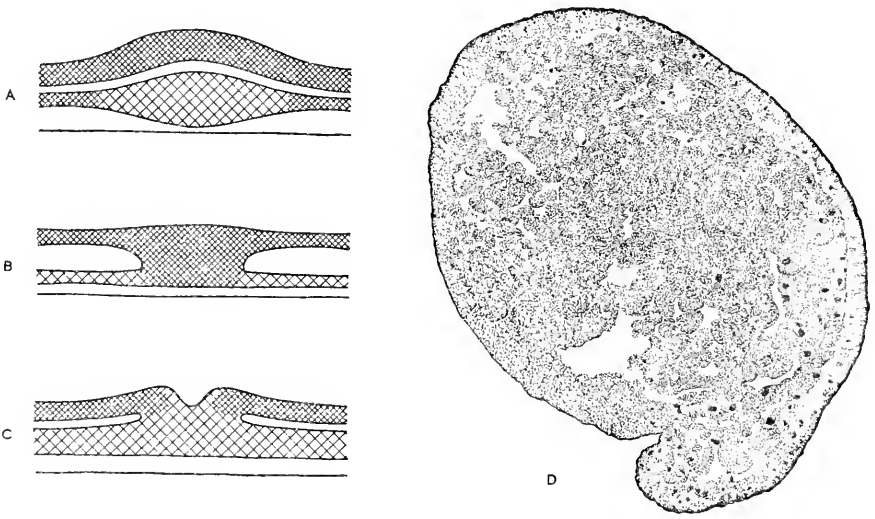


FIGURE 10.15

On the left, three diagrammatic sections through the head process (above) the node, and the streak (below) in a chick embryo treated with radioactive methionine. The shading indicates the relative concentration of the tracer, as judged from autoradiographs. (After Feldman and Waddington 1955.) On the right, an autoradiograph of a section through a newt gastrula cultivated in a solution containing radioactive methionine. The amino-acid has been incorporated into the proteins, and probably the nucleic acids, particularly in the nuclei of the blastopore region. (After Sirlin, 1955.)

the passage of radioactive phosphorus P^{32} into the RNA fraction of the egg (Kutsky 1950) as well as by histochemical methods. The latter show that the process is not uniform throughout the gastrula, but that the RNA is at first concentrated in the blastopore region. Histochemical methods which reveal protein containing –SH groups give an almost identical picture (Brachet 1944).

Less is known about the metabolism of different regions of the chick blastoderm at the time when the primitive streak is active as an organiser (Reviews: Waddington 1952, Spratt 1952*a*). Direct measurements of

oxygen consumption have so far failed to reveal any differences at the various levels along the streak (Phillips 1942), but Jacobson (1938) has shown that there is a rapid disappearance of glycogen during the invagination of the mesoderm, just as there is in Amphibia. Brachet (1944) finds that there is also a concentration of basophilic substances and of –SH containing proteins in the invaginating region, again as in Amphibia; in the chick, in which invagination occurs earlier at the anterior end of the streak, these substances are distributed in a gradient, decreasing towards the posterior. A similar gradient has been found for an indophenol oxidase (probably cytochrome oxidase) by Moog (1943). Rulon (1935) stained blastoderms in oxidised Janus Green, and showed that, under conditions of low oxygen tension, the dye was reduced fastest in the region of the streak; again there was a gradient decreasing from anterior to posterior.

Spratt (1952*a*, *b*) has recently studied such reducing systems in more detail, investigating the effects of aerobic as well as anaerobic conditions and varying the nature of the carbohydrate substrates available to the blastoderm. He made the very interesting observation that the node region shows a high rate of activity under a wider range of conditions than does the forebrain; Spratt suggests that we have here a chemical manifestation of the fact that the node is still undetermined and labile, whereas the forebrain has already entered on one particular and limited course of differentiation.

Feldman and Waddington (1955) have studied the incorporation into early chick embryos of radioactive methionine, which presumably gives an indication of the rate of protein synthesis. They found that the incorporation is particularly rapid in the node, and in the thickened ridges on each side of the streak (Fig. 10.15). There seemed to be some loss of the tracer from the newly invaginated mesoderm, which would suggest that a protein is broken down during the invagination process. The metabolism of this amino-acid seems to be of considerable importance, since administration of the unnatural analogue ethionine, which would be expected to interfere with the utilisation of methionine, causes considerable inhibition of development. Herrman (1954) has described rather slighter effects of certain other amino-acid analogues. Very marked inhibition, particularly of the streak and of the somites, is also caused by the purine analogue 8-azoguanine (Waddington, Feldman and Perry 1955). This would be expected to affect nucleic acid metabolism, and probably in this way has an influence on protein synthesis, for which RNA is certainly important.

This short summary is sufficient to make it clear that there are regional differences of metabolism within the avian blastoderm, and that, in

general, the most effective inducing region (the node) exhibits the highest metabolic activity. But it is even more difficult for the chick than it is for the amphibian to make any convincing suggestion as to which metabolic peculiarities are mainly responsible for endowing the organiser with its inducing capacity.

5. *What occurs during evocation?*

The study of the protein metabolism of the embryo is not only perhaps relevant to the activation of the evocator at the blastopore lip, but is even more likely to lead us into the centre of the problem of the nature of the biochemical events concerned in evocation. The differentiation of tissues certainly involves the appearance of tissue-specific proteins and it is probable that an alteration in protein metabolism is one of the most important results of induction. It is, however, extremely difficult to detect small differences in the protein constituents of cells and the analysis of the protein metabolism connected with early differentiation and determination is as yet in its infancy. New techniques will probably have to be elaborated before we can get very far. One of the methods now being explored is paper chromatography. When the proteins of the embryo are hydrolysed and the amino-acids assayed by this method no differences were found either between different stages of development or between different species (Holtfreter, Kozalka and Miller 1950; Eakin 1952). Using fresh unhydrolised tissues, Clayton (1954) has found considerable differences between tissues in fairly young mouse embryos but in these tissue differentiation had already proceeded well beyond the point at which evocator action occurs. Kutsky, Eakin, Berg and Kavanau (1953) have used similar methods in whole amphibian embryos and found a definite sequence of stages as development proceeds. It is probable that further work will show that this method of analysis can give valuable results in the analysis of different regions of the embryo.

Another method which is beginning to be used depends on the incorporation into the proteins of amino-acids labelled with radioactive isotopes. Eakin, Kutsky and Berg (1951) have found that such amino-acids are incorporated more rapidly into dorsal halves than in the ventral halves of the gastrula; and probably this means that proteins are being synthesised more rapidly in the blastopore region. Sirlin and Waddington (1954) have also found that the rate of incorporation varies from tissue to tissue in the early neurula. These two authors, and also Ficq (1954) discovered the interesting fact that the amino-acids are incorporated more rapidly into nucleus than into the cytoplasm in these early stages of development. This may indicate that protein synthesis is actually pro-

ceeding in the nucleus itself, or it may merely mean that amino-acid turnover is more rapid in the nuclear than in the cytoplasmic proteins. As mentioned above, the nuclear uptake begins first in the region of the blastopore, and rapidly spreads into the neural plate. It is probable that in the next few years a great deal of information will become available by this method.

A third method by which a direct approach has recently been made to the problem of protein synthesis as the basis of determination is the use of immunological techniques. Much of the older work in which such methods were used on embryos (Review: Needham 1942) was concerned with the development of species specificity; it is a well-known, though remarkable, fact that tissues of young embryos of quite different species or genera of Amphibia and birds may be grafted together and prove themselves mutually compatible until late stages in development. Even grafts between early embryos of fish and Amphibia or mammals and birds survive for a period which may be quite considerable. Nevertheless recent workers have been successful in demonstrating the existence of organ-specific antigenic substances in very early stages of development, and in using immunological methods to study the changes in such substances as development proceeds. References to most of the recent literature will be found in Woerdeman (1953), Cooper (1950) and Clayton (1953) for the Amphibia and Ebert (1952) for the chick.

Most authors have prepared anti-sera by injecting (into rabbits) minces or extracts of tissues taken from adult animals, and have then tested extracts of embryonic organs to discover whether they react with the anti-serum; if they do, it is concluded that they contain an antigen similar to, or the same as, that in the adult organ. However Clayton (1953) and Flickinger and Nace (1952) prepared their anti-sera by injection of embryos, or parts of them. Clayton made anti-sera against (1) early gastrulae of *Triturus alpestris*, (2) ectoderm of the early yolk-plug gastrula, (3) archenteron roof of the same stage, and (4) whole tail-buds. When extracts of an embryonic organ were to be tested against these, the whole anti-serum could be used for the test, or it could be first subjected to a process of fractionation by the method of selective absorption; for instance, anti-tail-bud serum can be allowed to react with an extract of gastrula until all the antibodies against antigens present in the gastrula are removed, so that only the antibodies against antigens which arise between the gastrula and tail-bud stages are left. Using this method, some important, though still tentative, conclusions were reached; the reason for hesitation in putting them forward as fully proved is that immunological methods, although extremely sensitive, cannot of course

be guaranteed to detect the most minute traces of antigens, and it is possible that substances which seem suddenly to appear during development may have been present in undetectable amounts at earlier stages.

Bearing this caution in mind, Clayton's results were as follows. She was able to detect six different antigenic substances (proteins?) in the blastula to neurula stages. She divided these into (1) a *C* or common group, which occur in both the ectoderm and mesoderm of the gastrula. Part of this fraction (*C*) is already present in the blastula, but another part (C^1) arises after or during gastrulation, and is presumably synthesised at that time. There are also (2) an *E* or ectoderm group and (3) an *M* or mesoderm group, which are confined to the ectoderm or the mesoderm respectively. Again in both these groups there are some components (*E* and *M*) which occur in the blastula, and others (E^1 and M^1) which arise during gastrulation. In the blastula, the *E* and *M* antigens are thought to exist side by side, only becoming segregated into different tissues as gastrulation proceeds. Similarly, the CC^1 EE^1 antigens of the gastrula ectoderm become sorted out between the neural plate and the ectoderm of the neurula. The former probably receives mainly CE^1 and the latter C^1E, but the identifications cannot yet be made with certainty. New antigens also appear during neurulation, e.g. a fraction named *Y* (Fig. 10.16).

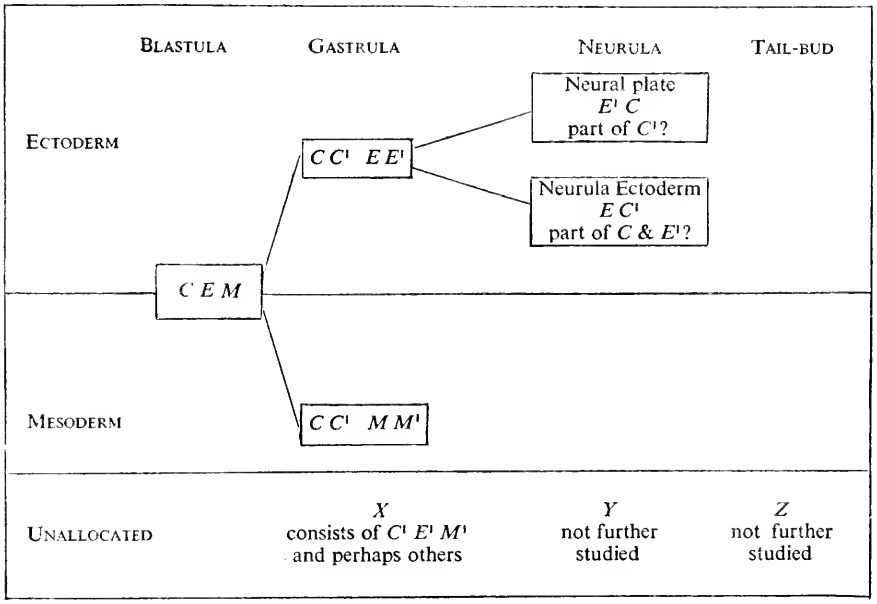


FIGURE 10.16

Diagram of the antigenic fractions found in various regions of the newt embryo. (From Clayton 1953.)

Thus although these studies are as yet in their infancy, they have already yielded considerable evidence that new proteins are being synthesised during gastrulation, and it seems likely that immunological methods will be a very powerful means of investigating the fundamental problem of protein synthesis during development.

It will be noted that Clayton found that the gastrula ectoderm contains certain antigens (*C* and C^1, and *E* and E^1) which in the neurula become spatially separated out between the neural plate and epidermis. Her data provide no direct evidence of whether they are already localised in the presumptive areas of the gastrula, but we know that differences in epigenetic behaviour (competence, and capacity for self-differentiation) are only just beginning to arise in gastrulae of this age, and it seems likely that the two groups of antigens, which later become separated, are both present in the same cells in the earlier stage. If this were true, it would be an important fact concerning the mechanism of induction, since we should have to suppose that the antigens characteristic of the neural plate are in some way destroyed or rendered inoperative in the developing epidermis, and vice versa. Some evidence that this may actually be the case can be found in the important studies of Ebert on the chick.

Ebert (1950, 1952) first prepared anti-sera against three adult organs, brain, heart and spleen. The most satisfactory method of testing the embryonic stages for reactivity with these was by adding the anti-sera to agar-albumen clots on which the young blastoderms were allowed to develop. At certain critical concentrations of the anti-sera, rather specific effects were produced on the developing tissues. The sera prepared against mesodermal organs (spleen and heart) tended to prevent the differentiation of mesoderm even at the primitive streak and early somite stages, while having less effect on the nervous system (except in so far as the latter was affected by the suppression of the inducing mesoderm). The two anti-mesoderm sera differed in that the anti-heart serum had a more drastic effect on the heart than the anti-spleen one. The anti-brain serum, at the critical concentrations, had little effect on the mesoderm but suppressed the development of the neural tube. We have then clear evidence that soon after their determination, and during the early phases of differentiation, the different tissues produce different antigenically-active chemical substances, presumably protein in nature.

In later work, Ebert (1953*b*) used sera prepared against the protein (myosin) of adult chick hearts to test various regions of the early blastoderm. He found that there was no reaction by blastoderms younger than the mid-streak stage. By that stage, the invagination of mesoderm has got properly under way, and the mesoderm is probably fully determined

to become mesoderm and nothing else; and it is then, at this very early stage, that the immunological techniques show that myosin is already present. In the next stage, of the long primitive streak, Ebert tested not only whole blastoderms but parts of them, as shown in Fig. 10.17. As far as the tests went, myosin was present in all the regions which contain presumptive mesoderm. By this stage, the individuation of the mesoderm

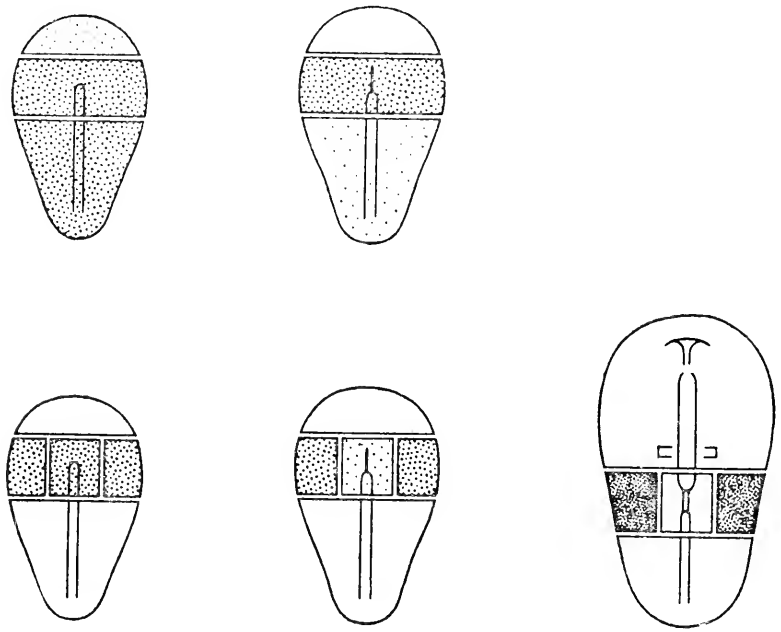


FIGURE 10.17

Parts of the chick blastoderm tested immunologically for their content of cardiac myosin. The intensity of their reaction with test sera is indicated by the density of dotting. Above, streak and head process stages cut into three strips. Below, the central strip cut into a middle and two lateral portions. (After data of Ebert 1953*b*.)

is beginning, and different parts of it are acquiring more specific properties (p. 187). By the head process stage, this regionalisation has gone some way; and, further, by this time the presumptive heart mesoderm will have been completely invaginated and moved out laterally to either side of the streak. Ebert finds, in accordance with this, that the myosin reaction has become very weak both in the posterior part of the blastoderm (posterior to the presumptive heart) and in the anterior part of the streak (between the two presumptive heart rudiments), and has disappeared

entirely from the anterior region of the blastoderm. By the next stage (head-fold and early neural groove) it has disappeared also from the anterior part of the streak, and is clearly becoming confined to the actual heart rudiments.

This beautiful series of experiments seems to provide clear evidence that a specific protein can be synthesised throughout the whole of an embryonic region (here the presumptive mesoderm) and then, as regionalisation proceeds within the region, disappear, or at least become undetectable, in all parts except the actual rudiment of the particular organ to which it belongs. It might perhaps be that its disappearance is illusory, and that it merely becomes concealed because it fails, outside its own rudiment, to increase as fast as it does inside it, or as fast as other substances are doing. But the evidence suggests that this is improbable; it seems more likely that the disappearance is a real one. If so, the fact is of capital importance for our understanding of epigenetic processes. It seems quite compatible with the hypothesis (advanced on p. 407) that in development we have to deal with systems of competing processes of such a kind that if one of them gets an initial advantage in a group of cells it will eventually run away with the whole system in that region. Provided that the synthetic processes are reversible, it would be possible for a substance *A* to be produced at an early stage, and then, as some other process leading to the formation of *B* pulled ahead, for the material which had gone into the *A* channel to be as it were sucked back and taken over by the *B* process, so that *A* disappeared again.

Another immunological investigation which has pushed back the detection of specific substances to an early stage, is that of ten Cate and van Doorenmaalen (1950). Using anti-sera against chick and frog adult lenses, they could detect a reaction with the embryonic lens at the stage when it is first becoming detectable anatomically as a vesicle or epidermal thickening.

Clayton (1954*b*) and Clayton and Feldman (1955) have recently introduced a new refinement into the techniques for recognising embryonic proteins by the use of antisera. The antibodies are coupled with either a fluorescent dye or radio-active iodine. When they are then allowed to act on sections of frozen-dried material, the position of the dye or of the radio-active material (which can be made visible by autoradiography) indicates the location of the corresponding antigens.

Besides these direct attacks by new techniques on the problems of protein synthesis in embryos, a considerable amount has been learnt about evocation by older methods. Brachet (1944, 1952*a*, *b*) has drawn attention to the importance of small granules which can be seen in the cytoplasm

in microscopic preparations. These he called microsomes. They can be shown, both by direct chemical methods and by their staining reactions, to be ribonucleo-protein in constitution. They have in general rather low enzymatic activity, but Brachet suggests that they may be the sites at which protein is synthesised. It is worthy of remark that according to a recent report, ribonucleic acid itself shows enzyme activity and is capable of breaking down dipeptides (Binkley 1951); such enzyme actions are frequently reversible and it may be that the nucleotide moiety of the microsomes plays a direct role in coupling together amino-acids to form proteins. The granules certainly increase greatly in number at the beginning of gastrulation, particularly in the organiser region and in the invaginating mesoderm; a little later they appear in large numbers in the developing neural plate. Brachet marshals a large body of evidence, which is in sum quite impressive although unfortunately most of it is somewhat indirect, in support of the hypothesis that these particles play an essential role in induction. For instance, if gastrulae are given a high temperature shock (about 36–37° C., i.e. some 2° C. below the lethal temperature) the gastrulation stops completely and no induction occurs; simultaneously the microsomes lose their nucleic acid. A very similar state of affairs occurs in hybrids between certain species of frogs, in which the disharmony between the cytoplasm and nucleus leads to a blocking of development at the gastrula stage; this evidence shows that the genes must be involved in the metabolism of the microsomes, a point which is of great general importance, as we shall see later (p. 382). In both the heat-treated and the hybrid gastrulae the rate of oxygen consumption ceases to rise from the time at which development stops. [Barth and Sze (1951) have shown that the summed oxygen uptake of a piece of organiser and a piece of gastrula ectoderm is higher when they are placed together so that an induction can occur than it is when they are kept separate. But it is not clear whether the extra oxygen is used for the actual evocation itself, or for the neural differentiation which the induced ectoderm undergoes.] It is rather surprising to find that these changes can often be reversed and development be started up again if a fragment is transplanted to a normal host embryo, which must be able to supply some essential substance lacking from the blocked tissues. The nature of the substance is obscure.

Again, Holtfreter (1945, 1948*b*) showed that when gastrula ectoderm is submitted to abnormal media (producing so-called sub-lethal cytolosis) there is a massive appearance of microsome-like bodies in the cytoplasm. Waddington and Goodhart (1949) made much the same observation when studying the mode of action of the steroid-like evocators. The location

of these substances in the cell can be revealed by their fluorescence when illuminated with ultra-violet. It was found that when applied to amphibian gastrula cells they become, in the first place, attached to lipochondria, that is, to cytoplasmic granules which contain considerable quantities of lipid as well as protein. In normal development, as Holtfreter (1946) showed, these granules break down at just the time when the microsomes are increasing in number during gastrulation and it seems likely that the microsomes are, in fact, produced by the dissociation of the lipochondria into their lipid and protein parts. Under the action of the steroid evocators the breakdown is accelerated. Finally, Pasteels (1951, 1953) has noticed a similar appearance of basophilic cytoplasmic granules in gastrula cells which have been centrifuged and thus caused to undergo spontaneous neuralisation.

All these observations provide considerable support for Brachet's suggestion that the microsomes are intimately involved in the reaction of the gastrula ectoderm to evocatory stimuli. They are probably in fact the site of the reactions *b* to *c* to *d*, etc. as postulated above (p. 197). This accords well with the evidence (pp. 90, 101) that centrifugable granules are important in determining the characters of the different regions in mosaic eggs, the gradients in echinoderms, etc.

Brachet also suggests that microsome-like bodies not only constitute the system which controls the competence of the reacting ectoderm but are also the natural evocator itself, that is to say the substance we symbolised as *a* in the scheme above. He brings forward several pieces of evidence in support of this. Firstly, he claimed (1944) that if dead tissues, capable of evocating, are digested with ribonuclease they lose their inducing power at the same time that the microsomes granules are destroyed; but more recent work (Brachet, Kuusi and Gothié 1952) has shown that this is not necessarily the case; it may be the protein, rather than the RNA which is effective. Secondly, he points out that in normal development there is a considerable accumulation of basophilic granular material between the invaginated mesoderm and the overlying ectoderm and although it is difficult to be certain, there is some suggestion that these particles are actually passed from the lower layer of tissue into the upper. Thirdly, Brachet stained living mesoderm with a vital dye, neutral red, which attaches itself mainly to the microsome granules. When this mesoderm is placed in contact with reactive ectoderm the induction takes place and at the same time the red colour is seen to pass into the reacting tissue. This might of course be a mere diffusion of the liberated dye itself. That possibility is rendered improbable by the observation that if a porous membrane, through which the dye molecules can pass, is placed between the

mesoderm and the ectoderm, no induction takes place and neither does the colour become transferred from one group of cells to the other. This seems to indicate that the transfer of dye is brought about by the passage of rather large particles which are not able to pass through the membrane, and thus supports the suggestion that particles of the size of microsomes are able to migrate from cell to cell. None of these observations are, however, as clear cut as one would desire to establish such an important conclusion as the intercellular migration of large microsome-like particles.

Niu and Twitty (1953) have recently made the important observation that if pieces of axial mesoderm are cultured in saline solution for some days, and small fragments of gastrula ectoderm then added to the culture, these may become induced to differentiate into neural tissue, even when they do not establish any direct cell-to-cell contact with the original mesodermal explant. The induction must be due to substances given off by the mesoderm into the culture medium. It is possible that these substances are relatively unspecific and act through a relay mechanism (as would, for instance, acids) but it seems more probable that we are really confronted here with a diffusion of the normal evocator itself. Preliminary spectroscopic study shows that the medium, after conditioning by the mesoderm implant, contains substances which may be nucleic acids; but the biochemical analysis is still in a very early stage. It has been mentioned earlier (p. 196) that Bautzmann (1929) had already shown that the body fluids of older larva exert an organiser-like influence on fragments of ectoderm isolated in it.

Some attempts have been made to get further information by the use of radioactive labelling. Waddington (1950*b*) showed that, if yeast is labelled by being cultivated for some time in solutions containing phosphorus-32, then dried and used as a graft in the gastrula, the radioactive material passes from the implant into the neuralising ectoderm. It is not clear however whether the phosphorus is carried by large complexes containing nucleic acids, or whether it is in the form of small groups such as the phosphate ion when it makes the passage from one tissue to the other. Ficq (1954) has also used organiser grafts labelled by cultivation in radioactive amino-acid solutions. She found that after a few days the radioactivity was no longer solely confined to the graft. It occurred in the evocated neural tissue, but it was also found in the neural system of the host embryo. It is therefore probable that we are dealing not with a straightforward diffusion of the radioactive material out of the graft into the surroundings, but rather with a selective accumulation by the most rapidly metabolising tissues of substances released from the graft itself, which become available throughout the whole embryo. Again it is un-

clear whether these substances are in the form of individual amino-acids or of larger, more complex particles. There is some evidence that, at later stages, after the blood circulation is established, different organs may give off substances which are complex enough to carry tissue specificity. Thus Ebert (1953) reports that if grafts are made on to the chorio-allantoic membrane of the chick, with fragments of spleen, liver or kidney from adult fowls injected with methionine-S^{35}, the radioactivity is found later to be specifically accumulated in the embryonic organ corresponding to the graft; that from spleen grafts goes primarily to the embryonic spleen, from kidney to the embryonic kidney, and so on. However, Sirlin and Waddington (1955) were not able to find any evidence of such organ-specific transfers of substances in the early stages of the amphibia, before the onset of circulation. They could also not confirm Ficq's observations of a passage of the tracer from a labelled organiser into the host neural tissue as well as into the induction. They point out that the tracer which gets into the induced tissues is mainly in the nuclei, and therefore gives no evidence for a passage of cytoplasmic microsomes. They suggest, indeed, that the tracer may actually be passing in the form of the free amino-acid, in which case it will yield little information about the mechanisms of induction.

Abercrombie and Causey (1950) have used radio-phosphorus to label regions of the chick primitive streak which were then used as inducing grafts into other blastoderms. They were able to distinguish the graft tissues fairly sharply from those of the host but their technique was not adequate to decide whether any minor spread of labelled compounds from the graft had taken place.

Thus the experiments using radioactive labelling have not yet given unequivocal evidence that bodies as large as microsomes pass from the mesoderm into the ectoderm during evocation, but neither do they refute the suggestion.

6. *Regionally specific evocation*

As was mentioned earlier, Spemann and many later authors have shown that the first invaginated, presumptively anterior, part of the mesoderm has a strong tendency to induce the anterior part of the nervous system, while the presumptively posterior mesoderm tends rather to induce trunk or tail regions. These phenomena present us with two rather different types of problem. On the one hand there is the question of whether the anterior and posterior parts of the organiser owe their specific effects to chemical differences in the evocators which they release. This is important for our present discussion, since if it were true it would be good evidence

that evocation is dependent on specific rather than unspecific stimuli. But however much we might discover about the chemical differences within the organiser, this would not provide any explanation of the way in which the reacting tissue comes to assume the specific and definite shape of a particular neural organ such as the forebrain, hindbrain or spinal cord, or of how the evocating substances come to be arranged in an orderly pattern within the mesoderm. This is the problem of morphogenesis and individuation. It will be discussed more fully later (p. 455). Here we shall deal only with the simpler chemical problem of the nature of the evocators involved.

There is considerable evidence (see Holtfreter 1951), that competent ectoderm of the gastrula has no particular regional properties and that no part of it shows any special tendency to develop either towards the head or the tail. Although Nieuwkoop (1947) fairly recently queried this, he seems to have had little good reason for doing so, other than the needs of a theoretical scheme he was putting forward. If therefore a process of induction shows a regionally specific character, this is to be attributed to the properties of the inducer rather than of the reacting material.

Early attempts (Lopaschov 1935*a*, *b*) to demonstrate the existence of chemically different evocators in different regions of the mesoderm were rather unsuccessful. However, shortly after this Chuang (1938, 1939, 1940) and Toivonen (1940) found characteristic regional differences in the inductions produced by implants of killed tissues from various organs of adult mammals (see also Hama 1944). The subject has since been rather thoroughly investigated by Toivonen (summary 1949, 1950). There is no close anatomical correspondence between the organ from which the implanted tissue is taken and the type of induction it produces. For instance, Chuang found that mouse kidney tended to induce parts of the brain, and *Triton* liver induced more posterior structures; while according to Toivonen, a guinea-pig kidney is a posterior inductor and guinea-pig liver an inductor of anterior parts. The distribution of the evocator substances in the adult body therefore seems to be rather haphazard.

Toivonen has made some progress towards finding out the chemical properties of the substances concerned. He concludes that there are two main evocators. The property of one is to induce anterior regions of the brain (the so-called archencephalon). This substance is relatively resistant to boiling, is soluble in organic solvents and easily dialysable. The second substance induces parts of the spinal column or the hindbrain (the deuterencephalon). It is very thermolabile, being destroyed by heating to 90° C. and is insoluble in petrol ether. The adult tissues as used in im-

plants nearly always contain mixtures of these substances, one or other predominating in particular cases; it is possible, for instance, to reveal the presence of small amounts of the posterior-inducing substance in guinea-pig liver tissue when the brain-inducing material is removed by thorough extraction with petrol ether.

The existence of these two different evocators seems rather thoroughly established by Toivonen's data. There are however several questions about them which still remain open. In the first place, should they really be regarded as specific inductors of particular regions, rather than of particular tissues? It is noteworthy that the archencephalon is the part of the nervous system which, in the normal embryo, is not accompanied by any mesoderm: and when posterior parts of the neural system are induced, some induced mesodermal structures always appear with them. One might in fact suggest (cf. Waddington 1952*c*) that the archencephalic evocator is a pure neural inductor while the spinal inductor is able to induce mesoderm as well as neural tissue, the anterior or posterior character of the induced organ depending on the proportion between the amounts of induced neural and mesodermal material. Toivonen (1953) has in fact recently found that alcohol-fixed bone marrow is a specifically mesodermal inductor causing the appearance of induced muscles, extremities, etc., unaccompanied by any induced neural tissue.

Another problem is the relation between these evocators from adult tissues and the factors active in the invaginating mesoderm of the gastrula. It has been known since the early work of Holtfreter (1934*a*) that the organiser material of the gastrula, when extracted with boiling water, alcohol, etc., retains its power to induce neural tissue but loses that to induce mesoderm. Barth and Graff (1943) showed that the same is true if the organiser region is freeze-dried. Waddington (1952*c*) re-examined the effect of slight heat treatment. The abolition of mesoderm-inducing capacity was confirmed and the evidence suggested that the neural-inducing capacity is not in any way regionally differentiated. Large masses of induced neural tissue sometimes form themselves into archencephalic vesicles (i.e. forebrain) ; and thus it seems not unreasonable to suppose that the heat-resistant neural evocator of the gastrula mesoderm is the same as the archencephalic evocator isolated by Toivonen from adult tissues. Lallier (1950) claims that the neural-inducing capacity of the gastrula organiser is abolished by treatment with formalin, but it is not clear whether this is true of Toivonen's archencephalic evocator. The mesoderm-inducing capacity of the living gastrula organiser also shows properties not unlike those of the spinal inducer postulated by Toivonen. In particular they are both heat labile. We shall see later when discussing

the individuation of the induced axis (p. 462) that there is evidence that the mesodermal inductor (or trunk inductor) acts after the archencephalic or neural inductor and probably antagonistically to it. There is not yet any corresponding evidence as to the time of action of the adult evocators, but in general it seems not improbable that Toivonen's adult evocators are at least very similar to, if not identical with, substances which are active during gastrulation.

Kuusi (1951, 1953) has studied the evocatory powers of various fractions (nuclei, microsomes, plasma, etc.) of the guinea-pig liver and kidney tissue. The results are not very easy to interpret but she comes to the tentative conclusion that the spinal (mesodermal) inducer is probably a protein while the archencephalic (neural) one may be represented by the microsomes.

A point which still requires considerably further study is the exact mode of action of the two classes of evocators. The gastrula ectoderm is normally two-layered, with an outer 'epidermal' layer and an inner 'sensory' one. In the normal induction of the neural plate, both layers become converted into neural tissue, but when abnormal evocators are used, their effect is sometimes confined to the inner sensory layer. Fujii (1944), comparing inductions produced by the coloured dorsal skin of the adult frog (which tends to evocate neural tissue) with those by the white ventral skin (which tends to induce mesoderm), raised the question of whether the two evocators act differentially on the two components of the ectoderm. Fujii's inductions were rather feeble, and his material not very convincing, but the problem would seem likely to repay further study.

7. *Competence*

The fact that it has been necessary to discuss at some length whether there is or is not any degree of specificity in the evocator(s) is sufficient to emphasise the great part played in development by the competence of the reacting tissues. What does this competence consist of, and what can be learnt about its behaviour? (Reviews: Waddington 1940*a*, Holtfreter 1951).

Competence, as was said above (p. 179), is a state of instability between certain alternatives. The gastrula ectoderm is competent with respect to the broad alternatives of epidermal, neural and mesodermal differentiation. Within each of these main categories there are certain subdivisions: for the first, true epidermis, lens placode, ear placode, etc.; for the second, the various regions of the brain, the spinal cord, neural crest, etc.; for the third, chorda, somites, nephros, lateral plate, etc. The state of instability

between these sub-alternatives persists longer than that between the main ones; thus pieces of tissue which have already become neural plate (and thus entered on one of the main alternative paths) may still for a short time be responsive to influences tending to change the region of the neural system which they will form. There is a considerably longer interval between the time at which the non-neural ectoderm is delimited and that when it is decided whether it shall become lens or ear. We have then a succession of competences in time.

The first study devoted to the causal relations, if any, between successive competences was made in the chick (Waddington 1934*a*). In that form, as we have seen (p. 182), the embryonic endoderm induces the formation of the primitive streak; does it also provoke the arising of neural competence in the ectoderm? If so, this competence should be absent in the *area opaca*, outside the region occupied by embryonic endoderm. It was found, however, that the *area opaca* reacts to organiser grafts just as well as does the *area pellucida*. The neural competence therefore seems to arise independently of the endoderm.

A similar investigation was made on the Amphibia (Waddington 1936). Pieces of ectoderm were removed from the gastrula before the organiser had acted on them, and were cultivated in isolation in salt solution until control embryos of the same age had reached the neural plate stage (i.e. had completed the primary organiser action); fragments of anterior neural plate were then implanted into them. The first fact that emerged was that the neural competence had been to a large extent, though not completely, lost. There is therefore an autonomous lapse of competence, although this is not as rapid as it would be in a complete embryo in which organiser action had taken place. Secondly, it was found that when the implanted anterior organisers developed into eyes, they were often able to induce the formation of lenses from the isolated ectoderm, in which lens competence must therefore have arisen independently of the action of the primary organiser. In more extensive experiments, Holtfreter (1938*b*) obtained similar results on the loss of competence, and showed that this is a gradual, not a sudden occurrence; as the ectoderm ages, the magnitude of the neural inductions diminishes, and one obtains more of the 'weaker' reactions such as the derivatives of the neural crest. The observation of Pasteels (1953) that centrifugation in the blastula stage often causes the appearance of mesodermal as well as neural tissues, while later only neural, and finally neural crest derivatives appear, is probably also to be explained by the waning of neural competence, but in this case the equivalence of the treatments applied at the different stages is not so certain, since the cells may change in their internal viscosity.

There is still very much to learn about the origin and loss of competence, and its quantitative intensity. It is, for instance, difficult to believe that all competences throughout development can arise autonomously, without dependence on previous inductive processes. There were indeed some indications in Waddington's experiments that the lens competence is not completely autonomous, since it seemed only to appear if the isolated ectoderm remained as a fairly thin sheet, and to be absent from thick solid masses of tissue (for discussion: see Waddington 1940*a*). Another very interesting problem is that implicit in the use of the phrase 'weaker reactions' above. Is the decision between the sub-alternatives dependent on the strength (i.e. intensity and/or duration) of stimulus? and can variations of this kind perhaps even tip the scales between the major alternatives of neural tissue and mesoderm? We shall return to this question in discussing individuation of the embryonic axis (Chapter XX).

It must not be overlooked that competence involves the capacity for self-individuation leading to the formation of a well-shaped organ. In the normal development of the neural system, the inducing archenteron roof undoubtedly plays an important part in the individuation of the neural system, but quite well-organised differentiation can occur even when the inducer is certainly structureless and unable to contribute to the result.

Although so little progress has been made with the embryological study of the waxing and waning of competence and the factors which bring it into being, there is another line of approach to which we may turn. Granted that competence is a state of instability in a complex system of reactants, what may we suppose these reactants to be? Now genetics has taught us that the characters which an egg develops are ultimately controlled by the genes contained in its nucleus. The various processes which may or may not proceed, according as the instability is resolved one way or the other, must therefore be gene-controlled; and the reactants which give rise to the competence must be the genes or at least factors dependent on the genes. Evidence of this may be seen within the organiser phenomenon itself. We have mentioned several times that organiser grafts may be active even if made between quite different species. If ectoderm from a species of Urodele with a large egg is grafted so that it comes to lie over the mesoderm of a small egg of another species, it is found that only a small neural plate is formed; there is an adjustment to the size of the inducing organiser out of which the evocator diffuses (Holtfreter 1935). But this is almost the only respect in which such an adjustment occurs. As regards nearly all other characters but size, the induced organs have the characteristics of the species to which the competent tissue belongs (for discussion:

see Baltzer 1950, 1952*a*). This is not so clear for the neural tissue, where there are no very obvious differences between the available species of Amphibia. But as we shall see, there are secondary organisers which act later to induce structures where such differences may be more marked. For instance, the mouth of an early frog embryo has proper teeth, whereas that of a newt does not; and if a newt mouth-organiser acts on frog ectoderm, the result is a frog mouth, complete with teeth (Spemann and Schotte 1932, Rotmann 1935). The inducing stimulus gives the order 'Mouth', and the reacting ectoderm carries out the order in accordance with its own book of procedure. The particular substances formed during the differentiation are determined, in the main, by the genetic nature of the competence. Another example of this is illustrated in Fig. 10.18.

It is worth noting that, as might perhaps be expected, Briggs, Green and King (1951) found no sign of any competence in non-nucleated amphibian cells, of the kind mentioned on p. 64.

It will be apparent from this discussion that the investigation of evocation has led us into the very heart of the complex metabolic life of the

FIGURE 10.18

A graft of newt ectoderm (pale) was made on to a toad embryo. Where the implant comes into the mouth region, it has developed a balancer, an organ which a newt embryo would possess in this region but which is quite foreign to the toad. Thus the implant has reacted in its own characteristic manner to the stimulus of the region. (After Rotmann 1941.)

cell. We are not dealing with a simple interaction between a single stimulating substance and a definite and delineated reactant. On the contrary, a choice as fundamental as that between epidermis and neural tissue involves the whole biochemical system of the cell; the protein synthesis, the ribonucleo-protein microsomes, the respiration and the genes. It is perhaps disappointing that the complexity of the system makes it impossible for us to reach a quick and definite identification of 'the evocator'. But in compensation we are acquiring a much more profound insight into the whole economy of epigenetic processes. Before we can advance any further, it will be necessary to discuss more fully the body of knowledge which genetics can bring to bear on the problem, which will be done in Part II of this book.

SUGGESTED READING

The classical papers on organisers are Spemann 1918, Spemann and Mangold 1924. Spemann's own summary of his work is in his 1938 book, Chapters 6–14.

For recent accounts of the experimental facts: Needham 1942, pp. 148–205 and 271–89: Lehmann 1945, pp. 203–350; Waddington 1952*a*, pp. 51–139. For biochemical aspects, Brachet 1944, Chapters 9 and 10, Boell 1948. Other valuable discussions, Brachet 1952*a*, *b*, Holtfreter 1948*b*, 1951, Toivonen 1950.

CHAPTER XI

EMBRYO FORMATION IN OTHER GROUPS OF VERTEBRATES

UNTIL fairly recently embryology has been in the main a comparative study. Its object has been conceived to be the derivation of a general scheme of development, of which the processes found in the different groups of animals could be regarded as modifications. In particular the diverse, but nevertheless obviously related, groups of vertebrates have provided fascinating material for study of the relations between different types of embryonic development. The wealth of available facts is indeed immense, and there are numerous textbooks devoted to it. Perhaps the best are Dalcq and Gérard's revision of Brachet's work and the recent book of Nelsen.

Comparative embryology of what may be called the classical kind was historically a derivative of, and theoretically should include, comparative anatomy. The comparisons instituted were between particular stages of the embryos of the various groups, each stage being considered as a static anatomical form. The results of such study are of great interest in relation to the general processes of evolution, though we consider them nowadays rather as raising problems than contributing materially to their solution. From the point of view on embryology which underlies the writing of this book, this aspect of biology remains rather peripheral, and since an adequate treatment of it requires the citation of an enormous number of detailed facts, we shall have to omit it and leave it in the hands of the comparative anatomists. The comparative method is still, however, a valuable tool of analysis. It can be applied not only to anatomical data but also to information of the kind with which this book has been mainly concerned. Such applications have only recently been attempted, but there are two types of comparative embryology which one must expect to increase greatly in importance in the near future. One is the comparative study of dynamic anatomical processes. Among the vertebrates there is a body of material which most urgently calls for treatment in this way, namely the active processes of gastrulation in the various groups which have been revealed by the use of vital staining or marking. Secondly, we now know enough about the causal sequences of epigenetic processes in the early development of the various groups to begin to institute comparisons between them, and to enquire what kind of alterations in these

causal mechanisms have been produced by the processes of evolution. The importance of the comparative method has been acknowledged in the main chapters dealing with the vertebrates by treating in immediate juxtaposition the two best-known types, the amphibian and bird. In this chapter the field of comparison will be widened to include the other vertebrate groups. These will not be treated so fully, since our purpose here is not so much to obtain a detailed and thorough knowledge of the epigenetic processes in each group but rather to provide a sketch of the salient features for purposes of comparison.

Each group will first be discussed in turn, and in the last two sections of the chapter an attempt will be made to draw the parallels and point the differences between them.

1. *Cyclostomes and other primitive fish*

The eggs of a representative of the cyclostomes, the river lamprey (*Lampetra* or *Petromyzon*) have been studied in some detail in recent years. The egg is about 2 mm. in diameter. Its cleavage is total and very similar to that of the Amphibia. It gives rise to a blastula which is also very similar to the amphibian. At gastrulation a small knob begins to protrude in the spherical embryo and the blastopore appears just below it. The blastopore is small and remains so throughout the whole period of invagination and one sees nothing resembling the yolk plug of the frog.

Weissenberg (1934, 1936) has studied the gastrulation by means of vital stained marks, and has derived a map of presumptive areas (Fig. 11.1). It shows a considerable resemblance to that of the Amphibia except that the axial mesoderm is, from the beginning, more concentrated towards the dorsal plane. According to Weissenberg, indeed, the presumptive

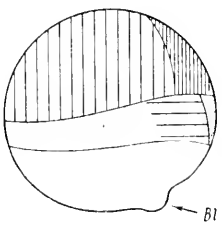


FIGURE 11.1

Map of presumptive areas in the young gastrula of the lamprey (cyclostomes). Close dots, chorda; spaced dots, mesoderm (somitic mesoderm with horizontal lines); close vertical lines, neural plate; spaced vertical lines, epidermis; white endoderm. The position of the mesoderm on the ventral side is uncertain. (Modified from Weissenberg).

mesoderm does not extend right round the egg but on the ventral side the presumptive ectoderm comes into direct contact with the presumptive endoderm. This situation, which would constitute a considerable difference from that of the Amphibia, has been questioned by Pasteels (1940) who points out that in the early neural plate stage, when invagination is still proceeding around the blastopore, this structure is undoubtedly surrounded by a complete ring of mesoderm, some of which is being invaginated over the ventral lip at this time. Although Pasteels' material was not sufficient to enable him to draw an alternative map of the presumptive areas in the early gastrula, he concludes that the mesoderm must in fact extend right round the egg between the ectoderm and endoderm. He suggests that the main difference between the gastrulation of the lamprey and the frog is that, in the former, the invagination of mesoderm over the lateral and ventral lips is delayed so that it does not begin until the main mass of endodermal material has already passed into the interior. The two processes in fact differ only in this comparatively minor matter of timing.

Some attention has also been paid to the causal embryology or epigenetics of these forms. It was shown as long ago as 1900 by Bataillon that if the first two blastomeres are separated each may give a complete embryo. The experiment has been repeated more recently (Montalenti and Maccagno 1935; Bytinski-Salz 1937*a*) and it has been shown that the situation is almost exactly the same as that revealed by constriction experiments in the Amphibia. Further, fragments from the dorsal lip of the blastopore can be grafted just as in newts (Bytinski-Salz 1937*b*; Yamada 1938). When they come in contact with gastrula ectoderm they cause the induction of a secondary neural axis and, so far as experiment has gone, repeat in every way the behaviour we have seen in Urodele material.

Unfortunately there has been little work by modern methods on other groups of primitive fish. Those types which have markedly telolecithal eggs and very unequal, but still total, cleavage would be particularly interesting as providing a transition to the higher forms, which are provided with very large masses of yolk and develop through the stage of a blastoderm. No vital stain experiments seem however to have been made on these transitional types. Ginsburg and Dettlaff (1944) have shortly reported a small number of experiments on the embryos of the sturgeon *Acipenser*. This has an egg, about 3 mm. across, which is rather amphibian-like in the cleavage and early gastrulation stages. Grafts of the blastopore lip showed that it behaved as an organiser and could induce secondary neural folds in a host embryo. The donors from which the blastopore lip had been removed developed no nervous system or axial organs, and the authors suggest that the presumptive materials for these

are confined to a smaller area than in amphibian eggs, although presumably located in a similar position.

The gastrulation process has been studied in some detail in the dog-fish, *Scyllium canicula*, a selachian and therefore usually considered more primitive than the sturgeons (Vanderbroek 1936). Its eggs, however, are more like those of teleosts (higher bony fish). They are provided with a large quantity of yolk, and the cleavage is partial, giving rise eventually to a blastoderm. This gradually spreads over the whole surface of the yolk, and as it does so invagination takes place along one sector of the circumference, after which the neural folds appear in the same position. The blastoderm is a fairly thick structure, and much of the endoderm lies from the beginning in the depths of it beneath the surface. This is particularly true of the extra-embryonic endoderm; some of the material which will form the endoderm of the embryo proper is at first on the surface, lining the position where the blastopore will appear. It is the first material to be invaginated. The disposition of the other presumptive areas is shown in Fig. 11.3. One point worthy of note is that the presumptive mesoderm does not extend right round the blastoderm on to the ventral side, but is confined to the dorsal region in the neighbourhood of the blastopore.

2. *Teleosts*

The eggs of teleosts are rather large, and contain considerable quantities of yolk. Although there is extensive variation from species to species in the relative mass of yolk and of living cytoplasm, the cleavage is always partial and leads to the formation of a blastoderm. The structure of the egg at this time is not altogether simple. At the animal pole there is a relatively thick plate of well-defined cells forming the blastoderm proper. Beneath this is a cavity, and underneath that again and extending for some distance around the blastoderm, the yolk is admixed with a fair quantity of cytoplasm and contains scattered nuclei; this syncytium is known as the periblast. Covering the whole surface of the egg is a thin cytoplasmic membrane which shows the properties of low permeability, high contractability, and lack of adhesiveness on its external face, which are also seen in the external membrane of the amphibian egg. It may be referred to as the 'coat', using the word employed by Holtfreter for the similar structure in Amphibia. Where it lies over the yolk outside the limits of the blastoderm, it is also known as the 'yolk gel membrane'. Its properties have been particularly studied by Devillers (1948) and Trinkaus (1949).

The blastoderm expands in area and after a time the sub-germinal cavity below it becomes well marked (Fig. 11.2). The maximum depth of the cavity which is roofed by the thinnest region in the blastoderm,

is not at the centre but is eccentrically based. This provides the first marked sign of bilaterality in the developing embryo. Vakaet (1953) has, however, found that the oöcytes of the fish *Lebistes* show a bilateral structure when they are growing in the ovary. It may well be, therefore, that the dorso-ventral plane in the blastoderm is foreshadowed at a much earlier stage.

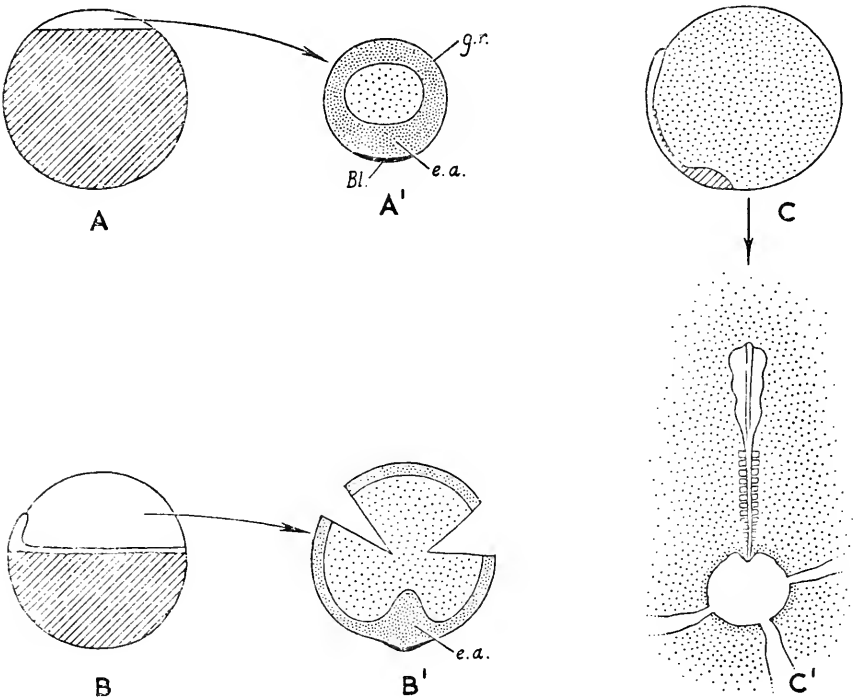


FIGURE 11.2

A, *B* and *C* are lateral views showing three stages in the spreading of the blastoderm of the trout egg over the yolk. *A'*, *B'*, and *C'* are views on to the blastoderm, which in the last two has been cut in order that it may be spread out flat. *Bl*, blastopore; *e.a.*, embryonic area; *g.r.* germ ring.

Gastrulation (Reviews: Pasteels 1940, Oppenheimer 1947) takes place by the in-rolling of the margins of the blastoderm. This occurs all round the circumference, but goes on most rapidly at one point, which becomes the site of embryo formation. The endoderm is the first tissue to be invaginated. Its presumptive area lies as a thin band in the margin of the blastoderm, much of it already below the surface (Fig. 11.3). It is probably concentrated mainly in the posterior region from which the embryo

will later arise, but its lateral and anterior extent is very inadequately known, and it may perhaps continue right round the whole margin of the blastoderm. Lying inside it, and the next material to be invaginated, is the mesoderm, which, according to Pasteels' work on the trout, certainly continues round the whole margin. Inside this again, that is to say, towards the centre of the blastoderm, is the presumptive ectoderm. The shape of the presumptive neural plate described by Oppenheimer in *Fundulus* is quite different from that assigned to it by Pasteels in *Salmo*, but the overall disposition of the areas is much the same in the two forms and their differences are perhaps such as we might expect from two not very closely related species.

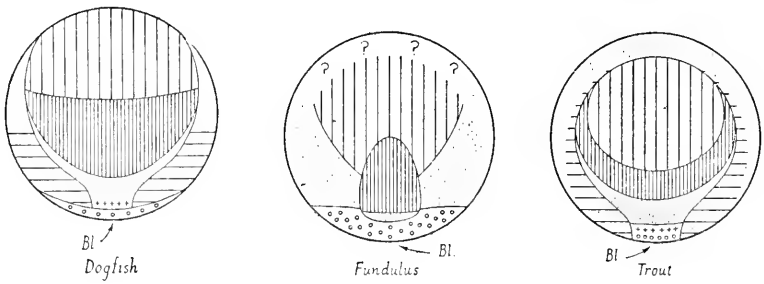


FIGURE 11.3

Presumptive maps of the early gastrula (blastoderm) in the dogfish (Selachia), and *Fundulus* and the trout (teleosts). Endoderm, circles; cephalic endoderm, small crosses; notochord, close dots; mesoderm, spaced dots (somites, horizontal lines); neural tissue, close vertical lines; epidermis, spaced vertical lines. (After data of Vandebroek, Oppenheimer, Luther, and Pasteels.)

While the gastrulation is proceeding, the blastoderm is expanding rapidly, so as eventually to cover the whole yolk. Its margin is often thickened, forming a structure known as the germ ring. After the expanding blastoderm has passed the equator of the egg, the germ ring contracts and acquires a superficial resemblance to a yolk plug before it covers the yolk mass completely. The extra-embryonic part of the blastoderm later becomes vascularised, and forms the yolk-sac by which the embryo absorbs its nutrients from the yolk. While the blastoderm is still expanding and gastrulation continuing at its margin, the embryo begins to appear (Fig. 11.2). The connection between the embryo and the thickened germ-ring caused many of the earlier embryologists to suppose that the two lateral halves of the embryo had originally been separated, one on each

side, as though the embryo had been split from tail to head and the two halves pulled apart. These two half embryos were then supposed to come together and fuse in the midline by a process to which the name concrescence was given. It can, however, be seen from the map of presumptive areas that this is not the case. There is always complete continuity from one side to the other across the midline. The presumptive areas are wider from side to side than the definitive organs will eventually be, but the movement from one situation to the other involves only a lateral contraction and longitudinal stretching, and not a moving together and fusion of two originally separate rudiments (Fig. 11.4).

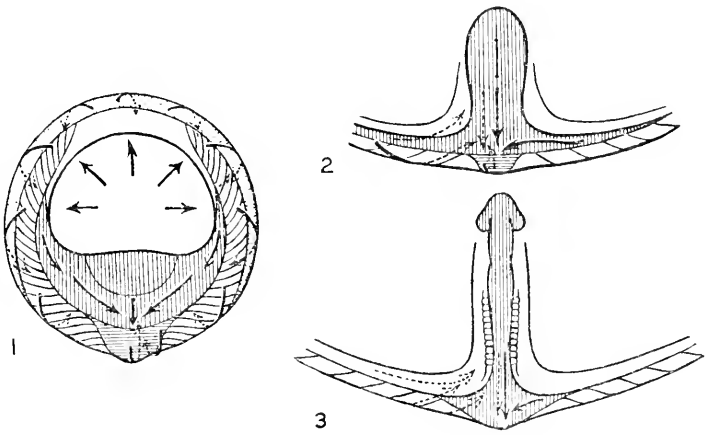


FIGURE 11.4

Gastrulation movements in the trout: *1* is an early stage, showing the whole blastodisc; *2* and *3* are stages in the formation of the embryonic axis. Movements taking place in the surface layer shown in solid arrows, those occurring below the surface in dotted arrows. (From Pasteels 1940.)

The mechanism of the gastrulation movements, and in particular of the spread of the blastoderm over the yolk, has recently been extensively studied by Devillers (1951*a*) and Trinkaus (1951). They both agree, in contradiction to certain earlier authors, that the spread of the blastoderm is not solely due to the contraction of the yolk gel layer. It seems probable that one of the essential factors is the activity of the periblast, which seems to have a spontaneous capacity to spread over the surface of the yolk. The blastoderm also has an autonomous tendency to expand, but it only does so over regions which the periblast has already covered and thus provided with a suitable substratum for it.

Quite a number of studies have been made on the epigenetic mechanisms which bring about the development of the teleost (Review: Oppenheimer 1947). They have dealt with several different species, none of which has as yet been fully investigated and the results do more to whet one's appetite than to provide a comprehensive account of the causal embryology of the whole group.

The earliest stage at which operations have been made is immediately after fertilisation. Such work goes back to the experiments of Morgan in the 1890s. The results of the most recent workers on *Fundulus* suggest that any one of the first two, or of the first four, blastomeres can give rise to a complete embryo. For example, Nicholas and Oppenheimer (1942) observed embryo formation in sixty-five cases out of seventy-two in which they had eliminated one of the first two blastomeres chosen at random. There seems to be little sign at this stage of an organisation centre which is localised as is the amphibian grey crescent. The existence of such a centre is, however, claimed by Tung and Tung (1944), using the eggs of the goldfish *Carassius*. They removed part of the uncleaved egg either by cutting it away with a knife or by pricking the future embryonic area and squeezing some of the cytoplasm out through the hole. They found that the earlier the operation was made after fertilisation, and the larger the part of the normal protoplasmic region remaining, the better was the differentiation. Fully normal embryos could be formed when the operation took place in about the first quarter of an hour after fertilisation and at least a half, or preferably more, of the protoplasmic region was left intact. In the abnormal and reduced embryos, mesodermal tissues sometimes differentiated rather well, but neural tissue does not appear to have been seen in the absence of accompanying mesoderm.

The same authors also divided the early blastoderm into two parts, cutting along the cleavage planes in either the 2-, 4- or 8-cell stages. They found that each part might sometimes give rise to a complete and normal embryo. In other cases there was one normal embryo and one mass of cells with no histological differentiation; and in some cases both portions gave this undifferentiated result. Two complete embryos can also be fused together, in stages up to the 16-cell stage. They sometimes produced complete, separate, twins, but occasionally one well-formed but oversized embryo to which it was clear that both eggs had contributed. All these results are exceedingly similar to those which have been found in the Amphibia, and might indicate a similar underlying mechanism, namely an organisation centre which becomes located in the plane of bilateral symmetry soon after fertilisation, which is essential for the development of the axial structures of the embryo, and which is capable of considerable

regulation, either when parts of it are removed or when two centres are fused together. But the critical evidence for such a centre is the fact that some blastomeres fail to give embryos. In view of the results of earlier work on *Fundulus*, it is perhaps unsafe to lay much stress on such essentially negative evidence.

In the stages immediately following this, a phenomenon has been noted which has sometimes been claimed to be a special feature of teleost development with no parallels in other groups. Oppenheimer (1934*b*, 1936*a*) found that blastoderms of the minnow *Fundulus*, if removed from the yolk at the 16-cell stage or before and cultivated in salt solution, failed to gastrulate and developed only into featureless balls of cells. A rather similar result was reached for the goldfish by Tung, Chang and Tung (1945). They found that if eggs were divided latitudinally at the 1-cell and 2-cell stage, blastoderms could not develop properly unless they remained connected with more than half the total quantity of yolk. From the 4-cell stage the requirement of yolk was less and from the 8-cell stage onwards completely isolated blastoderms could develop into embryos. Devillers (1947) found that the blastoderm of the trout was unable to develop in isolation from the yolk even when it had been removed as late as the blastula stage. On the other hand, in the pike *Esox* similarly isolated blastoderms continued developing quite well.

The explanation of these facts is not clear. As a matter of fact a somewhat similar phenomenon has been reported in the Amphibia. Vintemberger (1936) showed that if, in the 8-cell stage of the frog, the four animal cells are isolated, they are usually unable to differentiate any axial organs although they contain part of the presumptive posterior region of the notochord rudiment. If however the four cells are placed on a base consisting of a mass of endoderm cells from the blastula, they differentiate very much better. There are several different ways in which these results and those in the teleosts could be interpreted.

(1) It is a fairly general observation that early stages of embryos are more sensitive than older ones to the general injury produced by experimental operations. The increase in resistance usually continues at least until the main embryonic organs are laid down. It is noticeable, for instance, that it is easier to get good differentiation in tissue culture of chick blastoderms of primitive streak or later stages than of blastoderms taken from eggs in the first few hours of incubation. It may be that we are dealing merely with a general increasing 'toughness' of the embryos, which varies from species to species.

(2) Oppenheimer originally considered that the phenomena suggested the passage from the periblast into the blastoderm cells of some substance

of an organiser-like nature which was necessary for the initiation of gastrulation (see 1947, where she is already somewhat cautious about this hypothesis). Tung, Chang and Tung considered their results supported this. The boundaries of the cells, particularly of the periblast but also of the blastoderm, are not very sharply marked in early cleavage stages of teleosts, and it seems quite possible that the blastoderm continues to incorporate further cytoplasmic material for some time after the cleavages have begun.

(3) It may be, however, that what the yolk provides is not some organiser-like substance but rather a relatively simple essential nutrient. This possibility is supported by the fact that Devillers (1949) found that blastoderms of the trout, which are unable to differentiate in pure salt solution, will do so when glucose or similar substances are added to the medium. In a short note Trinkaus (1953) reports rather similar results with *Fundulus*.

Only if the second of these possibilities is the full explanation of the facts would we be confronted in the teleosts with a situation which differed radically from that in the Amphibia (since it is improbable that a similar process is the explanation of Vintemberger's results). At present the matter must remain open.

Experiments on the gastrula stage show that, in teleosts, the invaginating chorda-mesoderm acts as an organiser in a manner extremely similar to that found in the Amphibia and in the chick. This was demonstrated almost simultaneously by Oppenheimer for the perch and *Fundulus* (1934*a*, *b*, 1936*b*) and by Luther (1935, 1937) for the trout *Salmo* (Fig. 11.5). The parallel with the amphibian and bird organisers is extremely complete (see Review by Oppenheimer 1947). For instance, there is a similar type of regional determination by the different parts of the organisation centre, and evocation by dead tissues has been demonstrated.

It has been claimed (e.g. by Oppenheimer 1947) that the teleost ectoderm has some tendency to differentiate neural tissue without the intervention of the mesodermal organiser. Oppenheimer tends to interpret this in terms of an evocation by cytolysis, since in the abnormal embryos which gave evidence of such autonomous differentiation the cells were somewhat unhealthy. It appears however that the neural tissue was not completely without any accompanying mesoderm, but merely without accompanying differentiated chorda-mesoderm. The induction of neural tissue by mesoderm which does not include the chorda, and which may itself differentiate rather poorly, is well known both in the Amphibia and the chick, and it appears unnecessary to postulate anything more than this to explain the phenomena in the teleosts.

It appears probable that there is a considerable difference of a quantitative, if not of a qualitative, character between the teleosts and the Amphibia in the extent of regulation which can go on in portions of the blastula and gastrula separated from the main centre of the organiser region. Luther (1936) divided the pre-gastrulation blastoderm of the trout into four equal quadrants, each of which was then isolated by grafting it into the yolk-sac epithelium of an older embryo. All four quarters were found capable of differentiating into all the main embryonic organs, although these were usually somewhat chaotically arranged owing to the

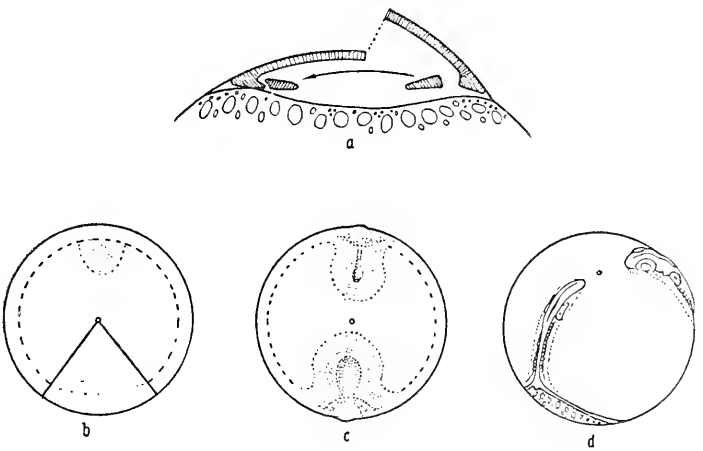


FIGURE 11.5

In *a* an early gastrula of the trout is opened, the tongue of invaginating material cut away and grafted into the opposite side of the blastoderm; *b*, surface view, showing the graft (dotted); *c* and *d*, stages in the development of the host and induced embryos. (From Luther 1935.)

abnormal mechanical conditions. Soon after the beginning of gastrulation this equality between the four sectors disappeared. The sector diametrically opposite the position in which the embryo will form soon loses its power to differentiate axial organs. There develops, in fact, a well-marked gradient, according to which the capacity for differentiation falls off from each side of the main embryonic region. In *Fundulus*, Oppenheimer (1953) finds rather better differentiation of embryonic regions (three successful out of six) than of extra-embryonic germ-rings (five out of seventeen) but the figures are hardly sufficient to establish the existence of a gradient.

In the Amphibia at similar stages (i.e. blastula and early gastrula) the capacity of isolated ventral portions to regulate and form a complete embryo is, of course, very much less than that just described for the teleost.

It is however not completely absent, as Dalcq and Huang (1948) have shown (p. 177). The teleost situation is, however, more comparable to that in birds at the time of endoderm formation, as described by Lutz (p. 183); there, again, the centre at which the embryo will form is not sharply distinguished from the rest, and far reaching regulation is still possible.

Devillers (1951) has studied the orientation in which embryos develop from portions of the trout blastoderm left in place on the egg or rotated in various ways. His results indicate that, in spite of the power of regulation which is spread throughout the whole blastoderm, the future embryonic region has already some slight degree of dominance both in the blastula and still more the gastrula stages, and it tends to dictate the orientation of the embryo. This again is very similar to the situation in birds.

The well-known evolutionary changes affecting the head skeleton of the fish have been discussed from the epigenetic point of view by Devillers (1950).

3. *Reptiles*

Passing over the Amphibia, about which enough has already been said, we come in our survey of vertebrate types to the reptiles. They have been studied, recently, in the light of our newer knowledge of other groups, particularly by Peter (1938) and Pasteels (1936–7, 1940). The main points which require notice concern the formation of the endoderm and the invagination of the mesoderm.

The eggs are large and full of yolk; cleavage is partial; and we meet once more a blastodermal type of embryo. When the presumptive areas are mapped, it becomes clear that the make-up of the blastoderm is radically different from that of the teleosts. Instead of the presumptive endoderm and mesoderm lying round the margin of the blastoderm, they are located within it, the edge of the plate of cells being wholly ectodermal. There is considerable variation within the group in the mode of endoderm formation. In the Algerian turtle *Clemmys leprosa*, Pasteels finds that the blastoderm at the end of cleavage is single layered. At a point, which lies not at the edge of, but within, the blastodermic sheet, a groove appears and cells of the single layer become pushed inwards to form the endoderm, which thus owes its origin to a true invagination through a blastopore. In other species, such as the chameleon and certain lizards, the blastoderm never becomes single layered, and much, though perhaps not all, of the endoderm is formed in place by a delamination similar to that which was described above (p. 155) for the bird embryo.

After the blastoderm has become two layered, and endoderm formation is complete or nearly so, the invagination of mesoderm takes place through the same blastopore. The tissues move to the blastopore, sink down through it, and are pushed forward in the shape of a tube which extends towards the anterior. This used to be known as the 'archenteric canal', but since, according to the vital staining experiments of Pasteels, it contains no endoderm but is made up wholly of mesoderm, it should, he suggests, be called the 'chorda-mesodermal canal'. Its roof will eventually become notochord, its sides somites, while its floor migrates further laterally to form the side-plates. At its anterior end the floor disappears at later stages, so that the canal leads from the blastoporal opening right through into the subgerminal cavity (Fig. 11.6).

Unfortunately, nothing whatever is known of the causal mechanisms of early reptilian development. The very slow differentiation of the eggs

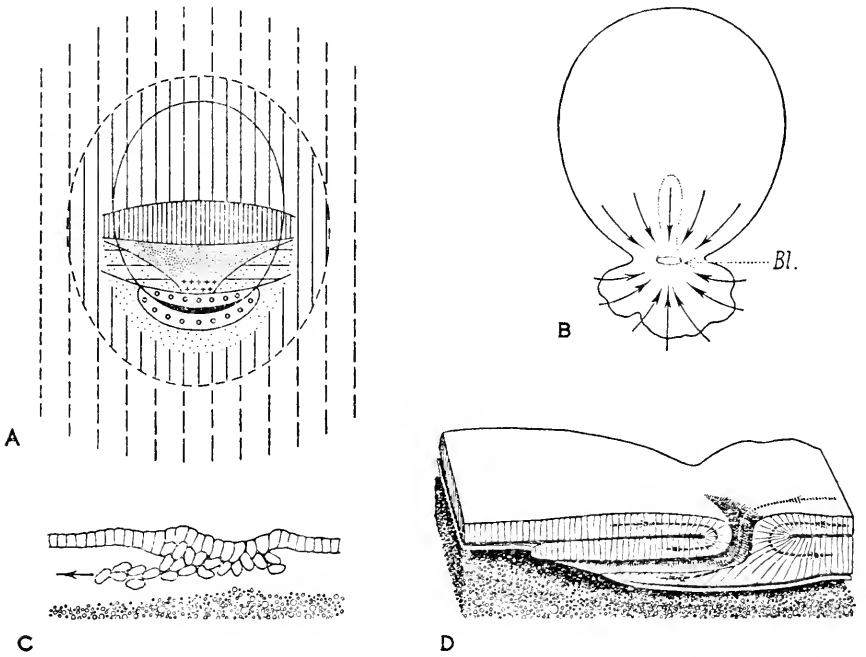


FIGURE 11.6

Gastrulation in the turtle *Clemmys*. *A*, map of presumptive areas, before the invagination of the endoderm (shading as in Figure 11.3, dashed vertical lines, extra-embryonic). *B*, movements of surface tissues towards the blastopore, from which the chorda-mesoderm canal extends. *C*, formation of endoderm from the blastopore at the beginning of gastrulation. *D*, the movements of tissues at a later stage through the blastopore into the chorda-mesoderm canal. (From data of Pasteels.)

makes them seem likely to be difficult experimental material, and no one has yet had the opportunity or the boldness to tackle them.

For the sake of continuity, it is worth while repeating two points about the avian embryo in relation to what has just been said about the reptilian. In the first place, endoderm formation is probably by delamination, as in turtles, not by invagination from a definite blastopore. Secondly, while mesoderm invagination is beginning, streaming movements take place from the posterior towards the anterior; thus instead of the mesoderm being formed from a circumscribed blastopore which leads in to a long forwardly extending chorda-mesodermal canal, it originates from an elongated primitive streak, from the anterior end of which there juts forward only a short extension of invaginated material, namely the head process.

4. *Mammals*

The major characteristic of the mammals (except for the most primitive group) is the adaptation of their embryos to intra-uterine life. Evolution has, in fact, been particularly active in the bringing about of changes in the early stages of development, and the group as a whole shows a very wide range of different conditions, which we shall only be able to sketch in very broad outline.

In the lowest group, the prototherian mammals or monotremes, such as *Echidna*, the egg is still reptilian in general configuration, cleavage is partial, and a blastoderm is formed. Flynn and Hill (1939, 1942) find that endoderm formation takes place mainly by delamination, though there is also some movement of isolated cells out of the upper layer into the deeper one.

In the higher groups (marsupials and true mammals), the form of the egg is completely different (Review: Nelsen 1953). It is small, and contains little if any yolk. Cleavage is total. Endoderm and mesoderm formation, however, occur by processes which are clearly modifications of those seen in the reptilian ancestors. The cleavage gives rise at first to a rather solid mass of cells, but a cavity soon appears amongst them. This is excentrically placed, so that the embryo assumes the shape of a hollow sphere, or blastocyst, to the inner face of which a thicker cluster of cells adheres at one place (Fig. 11.7). This is the 'inner cell mass', and from it the main embryo will be formed. The remainder of the sphere is extra-embryonic 'trophoblast', concerned with anchoring the embryo to the wall of the uterus; it is probably to be compared with the outermost parts of the reptilian and bird blastoderms, the *area opaca*. From the inner cell mass, the endoderm probably forms by simple delamination, though little is

known of this in detail and no vital marking experiments have yet been possible; there may well be some migration from the edges of the inner cell mass along the base of it towards the centre.

The mesoderm is formed from the outer surface of the inner cell masss. In this region the outermost layer of all, the 'enveloping layer' which is continuous with the main sphere of the blastocyst, either lifts away to leave a cavity (thus forming an amnion), or breaks down and disappears. The face of the inner cell mass thus exposed has in general an oval form in plan view, and on it there appears an elongated thickening, which is the primitive streak. In some forms, such as the rabbit, it is extremely like

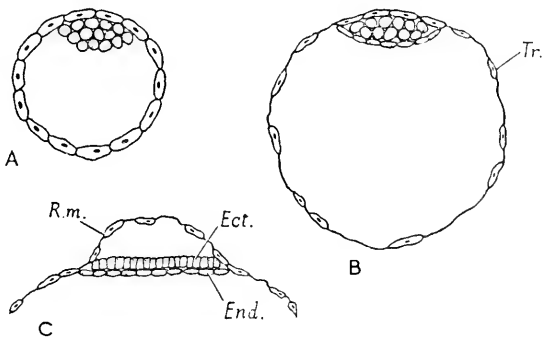


FIGURE 11.7

Early development in a mammal. *A*, section of blastocyst, with inner cell mass. *B*, later stage, the main part of the blastocyst becoming the trophoblast (*Tr*), the lower layer of the inner cell mass becoming arranged as an epithelium, the endoderm. *C*, the inner cell mass arranged as two epithelia, ectoderm and endoderm; above the latter the original outer layer has become elevated so as to form the amniotic cavity (*R.m.*, 'Rauber's membrane').

that seen in birds; in others, such as the mole and in man, the streak is shorter and there is a greater development of a chorda-mesodermal canal such as that found in reptiles. The invagination of the mesoderm has never been followed in detail by vital staining, but a few cinematograph films of rabbit blastoderms growing *in vitro* have been taken by Waddington, and a convergence of lateral material towards the streak from both sides has been seen; there is little doubt that the process is essentially similar to that of the bird and reptile. There are, of course, certain differences in detail. Thus in the rabbit the presumptive neural plate probably occupies a greater proportion of the embryonic area which corresponds to the area pellucida of the chick; and Waddington (1937) points out that the results of cultivation *in vitro* of posterior halves of rabbit embryonic areas

suggests that there is less longitudinal movement up and down the streak than in birds.

The first few steps have been taken towards a causal analysis of early mammalian development. Dalcq (1951*a*, 1952), and several of his pupils (cf. Jones-Seaton 1950) are engaged in a detailed study of the differential staining and other properties of the cytoplasm of the oocyte and early developmental stages of the eggs of a number of species. Their most important results so far are, perhaps, the demonstration that there is some degree of bilaterality in the structure of the oocyte and unfertilised egg; and that the distinction between the embryonic and extra-embryonic regions arises gradually, and is not fixed at, for instance, the first cleavage division as has sometimes been suggested (Fig. 11.8).

The latter conclusion accords well with experimental work. This is, of course, technically very difficult in mammals. It is first necessary, to operate on the young stages, to remove the eggs from the mother, then to divest them of the tough jelly which surrounds them or to operate through it, and finally to return them to some situation in which they will continue their development. Rodent eggs, which are easy to obtain, seem very sensitive to the removal of their membranes, and are difficult to keep alive in any situation except the uterus, although they can be re-transplanted back into another uterus with fair success (cf. Nicholas 1947, Willett 1953). The rabbit egg is in general tougher; the embryonic area at the primitive streak stage can be cultured *in vitro* for a time long enough for a fair amount of differentiation to occur. It is worth noting (cf. p. 231) that the older the embryo, the better it stands the conditions of artificial cultivation (Waddington 1937).

A little work has been done on the early cleavage stages. Nicholas and Hall (1942) succeeded in getting some development (up to just before the time the embryo makes its appearance) of one of the first two blastomeres, the other having been destroyed or removed. Pincus (1936) got rather similar results with the rabbit. Recently Seidel (1952*b*) had been much more successful. One of the first two blastomeres of a rabbit egg was killed by pricking with a glass needle, without removing the jelly layer. The egg was then injected into the Fallopian tube of another rabbit at a suitable stage in the reproductive cycle, and fully normal young were born. The evidence that one blastomere was really killed seems quite convincing, and the operated egg differed genetically (in colour) from the host, so that there is no doubt that the young animal was derived from it and not from an uninjured egg of the foster-mother. Only two such animals have so far been described; one was fully normal, but the other showed defects on one side, rather similar to those which are found in newts' eggs from

half blastomeres when the first cleavage furrow does not divide the grey crescent region into equal parts. One may conclude that the rabbit egg (and presumably that of other mammals) is certainly not of the mosaic type, but may contain a localised organisation centre comparable to the amphibian grey crescent; this would probably be related to the bilateral structures described by Dalcq. (For experiments involving the alteration of chromosome numbers, induction of polyploidy etc. in mammals, see Beatty 1955.)

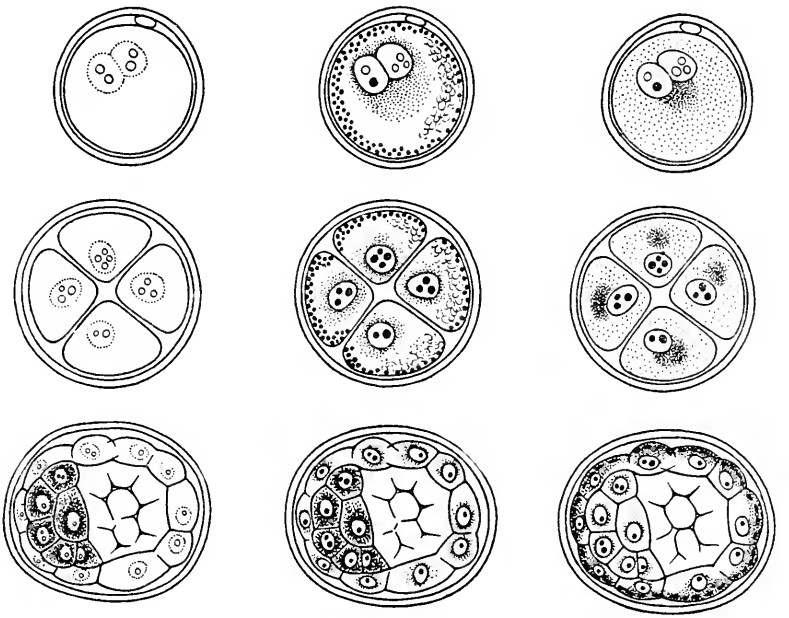


FIGURE 11.8

Three stages in the early development of the rat. Upper row, fertilised egg with pronuclei; middle row, four-cell; lower row, young blastocyst with inner cell mass. The eggs in the left column have been stained to exhibit alkaline phosphatase; those in the centre column RNA; and those in the right column mucopolysaccharides. (After original drawings of Dalcq.)

A few experiments have also been made at the primitive streak stage of rabbits although the material is technically very difficult to handle owing to its stickiness, transparency and resistance to cutting. Waddington (1937), failed to get any inductions by fragments of primitive streak transplanted between the epiblast and hypoblast in the manner used in the chick, but the material was certainly not extensive enough for this negative result to have much importance. On the other hand, the competence

of the rabbit epiblast to react to neuralising stimuli was demonstrated by cases in which such an effect was produced by grafts of chick primitive streak (Fig. 11.9); and rabbit primitive streak was also able to induce when grafted into the chick. It is highly probable, therefore, that the mammalian primitive streak is an organisation centre with functions similar to those of the same structure in the bird embryo. It was also shown that development of the posterior region is possible in the absence of the anterior end of the streak (Hensen's node), which some authors had considered to be an essential focus of embryo formation; again a result which parallels that in the bird. Törö (1939) has reported the induction of neural tissue in the rat by grafts placed in the amniotic cavity, but his

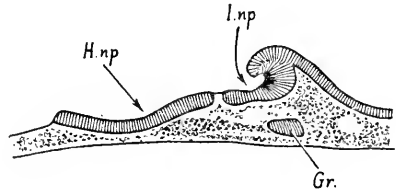


FIGURE 11.9

A small piece of chick primitive streak was grafted under the ectoderm of a rabbit embryo of the streak stage, which was then cultured *in vitro*. The graft has formed some neural tissue, and has induced a neural plate in the rabbit host. (After Waddington 1934.)

evidence, as published, is not very convincing, and it would be unexpected to find that tissues lying freely in such a cavity, in contact with the outer side of the ectoderm, could successfully induce; it seems more likely that he was dealing with embryos which were distorted as a result of the operations.

The results of direct operative experiments on mammal embryos have been supplemented by evidence of quite a different kind, which comes from an analysis by methods which have as yet been little used in other groups of animals, except the insects. A fairly considerable number of hereditary factors or genes are known which cause abnormalities in early developmental stages of mammals; perhaps their comparative frequency in this group is connected with the extremely rapid and radical changes which have led to the evolution of the group. The effects of these genes are often somewhat variable, and by a study of the variations it is sometimes possible to decide that one particular aspect of the abnormality is primary, and the remainder secondary consequences; from such arguments some insight into the causal sequences of epigenesis can be attained.

Dunn (1941) and in particular Gluecksohn-Schoenheimer (1949, 1953) working on the mouse, have made very important contributions in this way.

The first genes from which important embryological consequences could be deduced had their most obvious effects on the tail. The Brachyury gene *T* is a dominant, the heterozygous mice having short tails. The *T*/*T* homozygotes die before birth and in the embryos the whole posterior region of the body is missing (Chesley 1935). Detailed investigation showed that no notochord (or almost none) is ever formed in these homozygous embryos, and Chesley suggested that this was the primary action, the effect on other structures such as the nerve-cord being the result of secondary reactions of an inductive nature. This was confirmed, and the evidence made more convincing, when Gluecksohn-Schoenheimer investigated embryos of animals which were heterozygous both for *T* and for one or other of the genes t^0 and t^1. These embryos have normal-looking tails till an age of about eleven days, after which the tail becomes constricted at its base and degenerates, the young being quite tailless when born. Histological examination showed that even in the apparently normal tails of early stages, no notochord is present, although the neural tube, somites and tailgut are formed as usual. It seems then that the later degeneration is a consequence of the lack of the notochord, and some 'inductive' relation is indicated. It should be noted, however, that the relation is not quite that of evocation; even in the absence of the notochord, the neural tube is formed, presumably induced by the remainder of the mesoderm, but it is unable to persist. There is no exact parallel to this in other vertebrates, since the operative removal of the notochord, e.g. in Amphibia, does not lead to the regression of the neural tube, but only to a failure of its normal elongation and an alteration of its usual cross-sectional shape.

The homozygous t^0/t^0 embryos show more profound abnormalities. The whole endoderm tends to lift away from the rest of the inner cell mass some time before the beginnings of embryo formation are visible. The cells of the mass remain alive for some time, and some growth takes place, but there is no organisation of a primitive streak and no mesoderm appears; a day or two after the onset of the condition, the embryos die and are resorbed. Gluecksohn-Schoenheimer suggests that this may indicate that in the mammals, as in the birds, the endoderm may play an essential role in inducing the formation of mesoderm.

The same author has described a still more interesting gene, known as Kink. The heterozygotes show various spinal and tail abnormalities, and the homozygotes die at about nine days after fertilisation. Before death, a considerable amount of development has occurred, and the embryos

exhibit a most remarkable range of conditions, most of which can be considered as twinnings or duplications of various kinds, ranging from almost complete and separate twins to doublings of the main axis, of the heart, the allantois, etc. There are also many examples of single more or less complete embryos accompanied by ball-shaped lumps of unorganised tissue. Gluecksohn-Schoenheimer compares the structures found to those which result from the partial or complete separation of the first two blastomeres in Amphibia. If this comparison is accepted, and it seems quite convincing, one would have to conclude, firstly that the mammal egg at an early stage is capable of profound regulation (a point already clear from the occurrence of identical twins and directly confirmed by the evidence from blastomere separation mentioned above [p. 238]); and secondly that it possesses an organisation centre which is, or becomes, localised as does the grey crescent in the Amphibia or the posterior part of the endoderm in birds. The most plausible explanation of the action of Kink is that it interferes with the gradual regionalisation within the egg, by which the organisation centre becomes focused on to the dorsal side. Unfortunately the evidence does not help us to decide at what stage in development this takes place in mammals. In the armadillo, in which four identical twins are normally produced from each egg, the initiation of the four rudiments appears to occur rather late, in the blastocyst stage (Patterson 1913), and although one would now like to see the newly fertilised eggs of this form re-investigated by the methods of Dalcq, it is perhaps likely that the mammals are like teleosts in that their dorsal plane is not finally fixed till a relatively late stage.

It may be mentioned that all the mouse genes just mentioned (*T*, t^0, t^1 and Kink) are closely linked in the same chromosome; they may all be chromosome aberrations rather than true point mutations. Many other genes with generally similar effects (mostly not yet analysed embryologically) are known in the same chromosomal region. The causes for the association of this part of the chromosome with the primary organiser phenomena is quite unknown and present a very intriguing problem. Gluecksohn-Schoenheimer very tentatively suggests that possibly the spatial pattern of the genes in the nucleus may have some connection with the formation of the patterns of the early embryonic fields.

5. *Comparative geography of the presumptive areas*

In the past, attempts to compare the processes of gastrulation in the different classes of vertebrates have been made in terms of concepts which were derived from the static shapes which particular embryos may assume at certain points in their development. For instance, one tried to

homologise the structure of the blastoderm of the bird before endoderm formation begins with the hollow sphere formed by the amphibian egg at the end of cleavage; or one enquired how the chorda-mesodermal canal in reptiles is related to the amphibian blastopore. Nowadays we regard gastrulation as a co-ordinated series of foldings and movements by which the various groups of cells which result from cleavage are arranged into the three fundamental layers of the ectoderm, mesoderm, endoderm. What ought to be compared is the complete set of movements in one form with the complete set in some other form, rather than any particular instantaneous configurations which may be taken up during the process. The most manageable way of summarising the whole gastrulation of a particular group is provided by the map of presumptive areas at the late blastula stage, together with an indication of the directions in which these various areas will move.

When one looks at the maps of presumptive areas which have been described earlier, it is fairly easy to arrange them into a scheme in which they are brought into natural relations with one another. It is clear that such a scheme should start from a type of egg which is fairly small and has total cleavage, since the blastodermic forms of development must be secondary derivatives. Of the totally cleaving eggs, the most primitive fish (cyclostomes) and the Amphibia have maps which greatly resemble one another. The blastopore is placed low down on the vegetative side within an area of endoderm. Above this is a ring of presumptive mesoderm, which, certainly in the Amphibia and probably in the cyclostomes, extends right round the egg from the dorsal to the ventral side. Within this ring the presumptive axial mesoderm (notochord and somites) is concentrated somewhat towards the dorsal side, but extends much further laterally in the blastula than it will do after gastrulation is completed. The upper part of the egg is occupied by a cap of presumptive ectoderm, of which the region near the dorsal plane will become neural plate and the remainder epidermis. A very similar pattern is found in the protocordates (ascidians and *Amphioxus*), and we are probably safe in taking this as the basic type from which the others should be derived (Fig. 11.10).

There are two other main types to be fitted in. Between the cyclostomes and the Amphibia in the evolutionary sequence come the cartilaginous and bony fishes. These have a blastodermic type of development, and a characteristic feature of their presumptive map is that the margin of the blastoderm is made of presumptive endoderm or possibly mesoderm; the whole ectodermal area lies well inside the margin. The other groups—reptiles, birds and mammals—are evolutionarily more advanced than the Amphibia. Their embryos also develop from a

blastoderm but the presumptive map differs radically from that of the fish in that the edge of the blastoderm is in this case constituted of ectoderm. The presumptive mesoderm lies inside the margin and when the endoderm occurs on the surface, as it does in certain reptiles, it is the most centrally placed area of all. As between reptiles, birds and mammals, there are obviously great similarities, such differences as are found being dependent on the extent to which the endoderm is formed by delamination or is

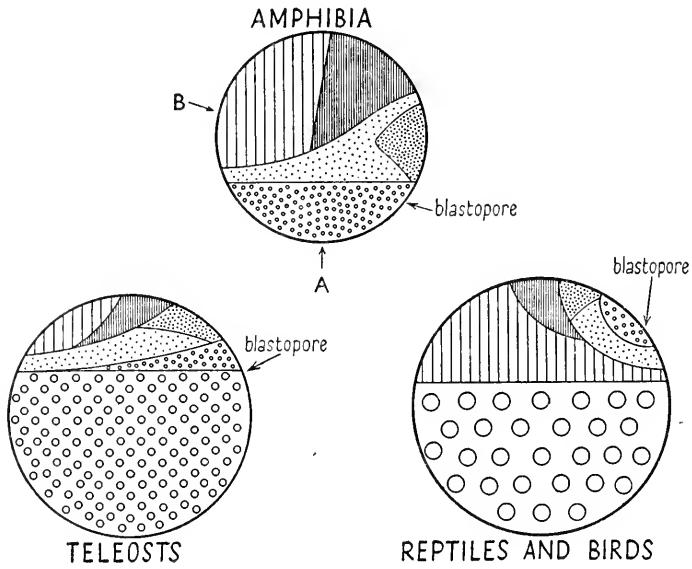


FIGURE 11.10

Maps of presumptive areas. The large circles in the teleost and reptile maps represent yolk; other conventions as before. In the amphibian map, *A* represents the point at which the yolk would have to be inserted to give the teleost map, and *B* that required to give the reptile-bird map. (From Waddington 1952.)

originally at the surface, and on the degree to which the site of mesoderm invagination is drawn out into a primitive streak or concentrated into a chorda mesodermal canal.

The movements in the eggs of all the groups are rather similar in general type. There are movements of expansion in all directions, affecting particularly the ectoderm. There is a 'dorsal convergence', in which tissues become narrower from side to side and simultaneously elongated, and do this the more intensely the nearer they lie to the dorsal plane. Again, there are tendencies for invagination, that is, for certain tissues to plunge inwards from the surface and continue their movements at a lower level. Finally,

in the blastodermal types, we find delamination, that is, a process by which an original single thick layer of tissue becomes rearranged into two thinner separate layers. Until we know more of the mechanical causation of the movements, it is not possible to make any further meaningful comparisons between them.

The main aspect of the comparative scheme which one would like to understand more fully is the relation between the two sorts of blastodermal development. Two main ways of regarding this have been proposed. Dalcq (1938) and Pasteels (1940), who were the first to discuss the matter at all fully, were content to accept an irreconcilable duality within the evolutionary system of the vertebrates. According to their scheme, the teleost map should be regarded as derived from that of the Amphibia by the insertion of a large mass of yolk near the vegetative pole, while the bird-reptile map is derived by inserting the mass of yolk somewhere within the area of presumptive epidermis (at point *B* in Fig. 11.10). This is the simplest and most straightforward scheme, but the idea of such drastic qualitative differences of egg structure within the vertebrates is not entirely satisfactory.

Waddington (1952*b*) has suggested that one ought to take into account not only the disposition of the presumptive areas on the egg surface but also their arrangement in depth. The blastodermal types might be derived from the amphibian arrangement, not by opening the latter at some point and spreading it out so that it can sit as a cap on top of a large mass of yolk which is inserted in the hole, but rather by imagining that the amphibian blastula becomes yolk-free, and then that the whole sphere is squashed down on to the surface of the separated yolk mass (Fig. 11.11). If, when the spherical blastula is distorted in this way, the line along which it folds (the 'primitive edge') lies wholly within the presumptive mesoderm, then the blastoderm will have a mesodermal margin and the whole of the endoderm will already be beneath the surface before gastrulation begins. If the primitive edge on the dorsal side lies a little lower, within the endodermal region, some of the dorsal margin of the blastoderm will be endodermal. According to the exact position of the line, one can easily arrive at the conditions seen in the Selachia or teleosts. Again, if the primitive edge lies higher on the amphibian map, it will, particularly on the ventral side, cut into the presumptive epidermis and the blastoderm will have an epidermal margin in that region.

In order to explain the fact that the whole of the blastodermal margin in birds is ectodermal, we should have to postulate an expansion of the presumptive epidermal area in addition to the flattening of the spherical blastula. This makes the scheme more complex than that of Dalcq and

Pasteels. Its main merit is that it gives an example of one way in which it is possible to envisage both the fish and bird blastoderms as two modalities of one general process; and further, it draws attention to the need to consider the disposition of the presumptive areas in depth as well as on the surface. It is most desirable that we should find out more about the location of the presumptive areas in the extremely yolky types of amphibian eggs, particularly those of the Gymnophiona. In these, much of the endoderm seems to be beneath the surface before gastrulation begins and to become distinct by a process of delamination, which would seem to accord better with Waddington's scheme than with the Dalcq-Pasteels' one. Gastrulation in these eggs has, however, not been studied since Brauer's work in 1897.

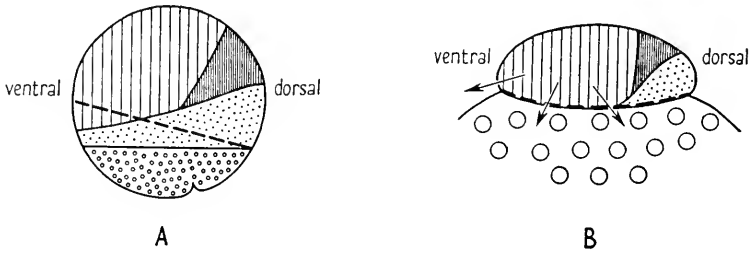


FIGURE 11.11

A, the basic vertebrate map, with a line of folding ('the primitive edge') indicated by a broken line. *B*, the blastula squashed down on to a mass of yolk, having folded along the primitive edge. If such a deformation is to produce the bird map, there would also have to be an expansion of the epidermal area. (From Waddington 1952.)

6. *Comparative causal embryology of vertebrates*

The various types of vertebrate eggs, different in many respects and yet all anatomically related in the ways we have just discussed, provide a wonderful opportunity for studies on the kind of alterations in epigenetic mechanisms which evolution can bring about. There is no doubt that one ought eventually to envisage evolution not as a process which merely causes modifications in the structure of adult animals, but rather as one by which the causal sequences of development have been changed. These changes have affected even the basic mechanisms which operate in the early stages of development. Dalcq (1951*b*) has referred to mutations which affect these stages as 'onto-mutations'. Little is known in detail about them from the genetical side, though many of the factors which are labelled (and usually dismissed) as 'female-steriles' probably fall into this category

(cf. p. 135). The comparative study of the epigenetic systems in different groups of vertebrates gives an opportunity to see the kinds of effects which such gene changes produce.

The first noteworthy epigenetic event in the development of vertebrates is the fixing of the plane of bilateral symmetry. In the Amphibia, as we have seen, the future organisation centre normally becomes located soon after fertilisation and is sometimes visible as the grey crescent. In the other primitive non-yolky type of vertebrate egg, that of the cyclostomes, Montalenti and Maccagno claim that the situation is very similar. In the protocordates (ascidians and *Amphioxus*) the dorso-ventral plane is also fixed definitely at an early stage. In both the ascidians and amphibians there is indeed some evidence that the dorso-ventral plane is at least foreshadowed before fertilisation occurs. In the latter the position of the plane is at first labile and only gradually becomes definitely determined. By the time cleavage is completed and the blastula stage reached, the dorsal plane is firmly determined and it is difficult to cause a new dorsal region to appear elsewhere when the egg is divided in two. Such powers of regulation are, however, not completely extinguished until considerably later (p. 177). The results obtained by Bytinski-Salz on the cyclostomes suggest that the same is true in that group.

In the blastoderms of both the teleosts and the birds the situation seems to be rather different. We have some evidence (Vakaet 1953) that the growing oocyte of the teleost has a bilateral structure, but in the developing embryos the position of the dorso-ventral plane is quite easily alterable until a late stage of the blastula, as is shown by the results of Luther in teleosts and Lutz in birds. It seems probable that the original bilaterality in the oocyte is swamped by the great flood of yolk which is laid down, and that a bilateral structure is only gradually and slowly re-established. It is true that Tung and Tung (1944) argued that, in the goldfish, an organiser-like region beomes located on one side of the egg shortly after fertilisation, and thus endows it with a bilateral symmetry, but it is doubtful if their experimental material was adequate to sustain this conclusion (p. 230). It seems likely therefore that we have to accept a difference in timing as between the holoblastic and blastodermal eggs, the dorso-ventral plane being determined much later in the latter.

In the Amphibia the agent which determines the dorso-ventral plane was the inner yolk mass. In the teleosts it seems to be the periblast. It is difficult to say how far these two can be considered as in any way comparable, either in their anatomical derivation or their mode of action. In both groups what is determined is, in the first place, the invagination of endoderm; and hard on the heels of this comes the mesoderm, which

proceeds to be invaginated around the same focus. In birds we have no direct evidence as to the mechanisms which determine the posterior end of the endoderm, which is the region that takes the lead as soon as the dorso-ventral plane is fixed. In this form, however, another stage is interpolated into the normal sequence, since there is something of a pause between endoderm formation and the start of invagination of mesoderm at the primitive streak, and we find that the endoderm exerts a causal influence which determines the site of mesoderm invagination. The apparently straightforward, follow-my-leader behaviour of mesoderm towards endoderm in the lower groups has, therefore, been expanded in the birds into a cause-effect sequence.

We still know too little about the epigenetics of mammals to fit them with any confidence into the scheme. There is evidence (Dalcq, p. 238) that the oocyte and newly fertilised egg have some degree of bilaterality of structure. Seidel, as a result of his separation of blastomeres, argued that there is a localised organisation centre. This result, however, was based on only two sets of operations and is exceedingly tentative. On the other hand, the evidence suggests that the formation of identical twins may occur at much later stages; and on the whole it seems probable that in mammals, as in birds, the final determination of the dorso-ventral plane occurs rather late.

It seems probable that, in all the vertebrates without exception, determination of the dorso-ventral plane fixes, in the first place, the location of the endoderm and, secondly, that of the mesoderm, but that the ectoderm remains quite indifferent till it is acted upon by an organising influence coming from the mesoderm. Grafting experiments have given direct proof of the inducing power of the presumptive mesoderm in cyclostomes, teleosts, Amphibia and birds and the demonstration is only slightly less clear-cut in mammals. In all groups there is evidence that at the time the mesoderm starts to be invaginated the fate of particular regions of it can still easily be altered, but that, as invagination proceeds, it is affected by a process of regionalisation so that different areas become determined, not only as to the tissue into which they will develop (as notochord, somites, nephros, etc.), but also as to their position on the anterior-posterior axis (brain, spinal cord, tail, etc.). There is some not very compelling evidence (p. 187) that in birds the presumptive forebrain has a tendency to develop into neural tissue independently of any induction by mesoderm. If this is so it may be connected with the fact that, in this group, the determination of the site of mesoderm formation (the primitive streak) is a comparatively long-drawn-out process caused by the endoderm. In other groups the development of neural tissue seems to be completely dependent

on induction, and the whole responsibility for the appearance of the main embryonic axis can be attributed to this process and to the self-individuation (i.e. regionalisation) of the mesoderm.

It seems therefore that all the groups of vertebrates employ essentially similar epigenetic mechanisms. The main changes which have occurred within the group appear to be: (1) The swamping of the initial structure of the oocyte by the enormous quantities of yolk laid down in fish and birds, and a consequent postponement of the time at which the plane of bilateral symmetry is finally determined, and (2) the interpolation of a new causal relationship between endoderm and mesoderm in birds (and possibly in reptiles and mammals) in consequence of the difference in the time of invagination of the two layers.

SUGGESTED READING

Needham 1942, pp. 320–30; Dalcq 1938, pp. 7–58. For comparative gastrulation, Pasteels 1940, Waddington 1952*b* or 1952*a*, pp. 39–50. For teleosts, Oppenheimer 1947; for mammals Gluecksohn-Schoenheimer 1949, Dalcq 1951*a*.

CHAPTER XII

ORGAN DEVELOPMENT IN VERTEBRATES

IT is clearly impossible, in a book of this size, to do more than sketch in very broad outline the extremely complex processes by which the details of the adult anatomy come into being. There are, moreover, already many excellent accounts of them; for more extended comparative treatments, Dalcq and Gerard's revision of Brachet (1935), and particularly Nelsen's recent work (1953) can be recommended.

In the present chapter, an attempt has been made to single out those aspects of organ development in which new advances have recently been made, or which are particularly important in connection with various general principles of development; and two organs, the limbs and the kidneys, which provide very interesting illustrations of a number of points, have been dealt with in rather fuller detail.

1. *The general form of the embryo*

At the end of gastrulation, the amphibian embryo—often known at this stage as a neurula—is still a solid, approximately spherical object. The neural system runs along its dorsal meridian, at first in the form of an open neural plate, which however rapidly closes up into a neural groove and finally a tube. It also stretches considerably in the anterior-posterior direction, continuing that process of elongation which we have seen to be characteristic of the dorsal region through gastrulation. At an early stage in this stretching, the head and tail curl round the opposite ends of the egg, which has now become somewhat ovoid, so that the embryo seems to be on the point of biting its tail; but as the elongation becomes still greater, particularly in the more posterior regions, the dorsal axis straightens out again and the creature begins to assume the form of a tadpole, with a long thin tail stretching out behind its body. During all this time, there has been a tubular gut, formed directly by the invaginating mesoderm and endoderm, and opening out to the exterior through the blastopore, which lies at its posterior end in the region where the definitive anus will eventually be. At first the tube has a floor and walls of endoderm and a roof of mesoderm, but the lateral edges of the endoderm soon grow in from each side to meet in the midline and from then on the gut-tube is lined entirely by endoderm; we shall see that this situation is not so easily brought about in the flat blastoderm of the birds. The wall of the

amphibian gut is at first rather thin on the dorsal side, but very thick on the ventral, where the cells are still swollen by the large stores of yolk. From the exterior, it appears as though little is happening on the ventral side while the tail is growing out, but beneath the skin the first blood cells are forming, and the mesoderm is beginning to form a pulsating tube, the rudiment of the heart.

The chick embryo, when it reaches the neural plate stage, is not, to speak crudely, a solid lump like the amphibian, but is instead a flat circular plate, on which the embryo proper is linear in form, the remainder of the plate being no part of the final body but concerned only with the absorption and digestion of the yolk and with the respiratory exchanges between the embryonic blood and the outside air. One of the most striking features of the embryo proper is the very great difference in the stage of development reached by the anterior and posterior parts. In the Amphibia the whole neural plate folds up more or less simultaneously to form a neural tube, which stretches from the anterior right to the posterior end, where there is only a small region of 'tail-bud' at which new additions of neural tissue and mesoderm continue to be formed. In the chick on the other hand, at a time when the anterior neural plate has folded up into a completely closed tube in the head region, it is still only a shallow groove at the level of about the tenth somite, while posterior to this there is still a remnant of the primitive streak at which mesoderm is being formed and the primary organiser is active. Thus although differentiation progresses roughly from anterior to posterior in both forms, the differences are much more marked in the chick than in the frog or newt.

Fairly soon after the closure of the neural tube in the anterior region of the chick, one can see the beginning of a process by which the embryo proper eventually becomes separated from the remainder of the blastoderm. Along a crescent-shaped line in front of the head, the blastoderm is tucked downwards and backwards under the neural tube. The fold so formed is known as the head fold. As it gets deeper and is pushed further backwards, the head region is gradually left isolated on a projection above it. At a considerably later stage, when the embryo has some thirty or more pairs of somites, a similar fold (the tail fold) cuts under the posterior end of the embryo; and eventually these two folds each progress so far towards the centre of the body that the whole embryo is left attached to the non-embryonic blastoderm only by a narrow stalk (Fig. 12.1).

As the blastoderm is tucked downwards in the head fold, a pocket lined with endoderm appears on the under side of the head. This is the first rudiment of the gut to appear, and is known as the foregut, to distinguish it from the hindgut which appears later in connection with the tail fold.

Bellairs (1953, 1954) has recently studied its development in detail, with the aid of vital stained marks. The foregut opens posteriorly on to the space between the blastoderm and the yolk, and its posterior edge is a clearly defined landmark in early chick embryos. As the head fold is pushed further and further backwards under the embryo, so the opening of the foregut moves posteriorly; in fact it does so even faster than the head fold progresses, and it is probable that its movement is not as simply dependent on that of the head fold as appearances might at first suggest (Waddington 1952, p. 149). After the opening to the foregut has progressed some distance towards the posterior, the mesoderm in the edge of the fold develops into a hollow tube, which at first extends some distance along each side of the crescent-shaped opening. This tube is the

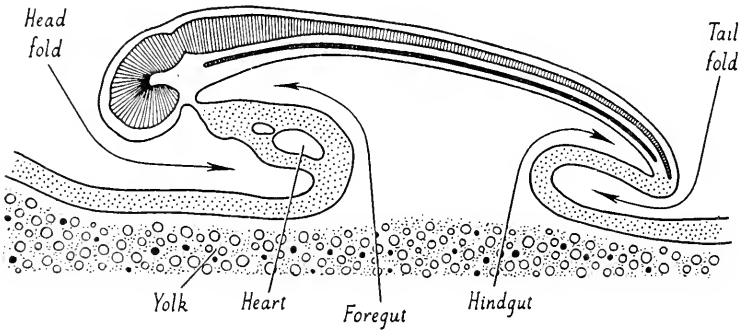


FIGURE 12.1
Diagrammatic longitudinal section through a chick embryo, to show the relation of the embryo to the yolk.

rudiment of the heart. It is really a double structure, since the mesoderm which forms the tube on the left side originally lay well out at the left of the blastoderm, and a considerable distance away from that which forms the tube on the right side; it is only the backward progress of the edge of the foregut, which takes place by a sort of zip-fastener process along the midline, which has brought them together.

This obvious doubleness in the early rudiment of the heart is one of the points in which the chick embryo seems to differ most radically from the amphibian type. Even so, the two systems are not completely different. We can relate them by the imagining what would happen if the central part of the chick blastoderm were wrapped round a spherical core of yolky endoderm; if the two presumptive heart regions were at the edges of this part of the blastoderm, they would come into contact on the ventral

side of the endoderm and no further folding would be needed to bring them together; we should in fact have arrived at the amphibian condition. The reverse of this can actually be achieved experimentally. Nieuwkoop (1946) has slit an amphibian neurula up the ventral midline, removed the endoderm and flattened out the mesoderm and ectoderm on a plate of stretched silk, so that it is forced into a sort of 'blastodermic' configuration. It then develops two heart rudiments, one on each side, which fail to fuse so as to form a single heart, since the embryo is not provided with the foregut-forming mechanism which brings this about in the chick.

In the stage immediately after its formation, the heart of the chick embryo continues to have an important influence on the general form of the body. In the first place, it becomes extremely large. This is necessary because it has to pump blood not only through the embryo itself, but for very much longer distances through the blood vessels which run into the non-embryonic blastoderm. It becomes so disproportionate to the body proper that it bulges out to one side. At the same time, the whole body rotates, so as to lie with its left side down against the yolk; and the head also arches round so that the anterior end of the brain is bent down towards the chest, with the heart protruding between them. It is the development of the heart and its associated structures which is the cause of most of these foldings and rotations, and if it is removed, the main part of the neural system remains straight, only the anterior tip of the brain bending downwards.

2. *The development of the head*

In both the amphibian and the bird, the neural plate is wider in the anterior than in the posterior. This is very clear in the newt, in which the whole plate is marked out on the surface of the egg at the time the blastopore is closing; it is definitely pear-shaped. As the edges of this area fold together to form the neural tube, the future brain, developing from the anterior end, has from the beginning a wider lumen than the hinder parts of the nerve cord. At first the tube remains open at its anterior tip, the small hole connecting the interior of the tube with the outside being known as the anterior neuropore; it closes gradually during the later development of the brain.

The comparatively slight initial difference in the width of the brain and of the main nerve tube soon becomes greatly increased by the appearance of swellings in the former. At first there are three, which form the primary brain vesicles which give rise to the forebrain, midbrain and hindbrain. Fairly soon the first and third swellings each become differentiated into

two, so that the brain comes to consist of five vesicles, whose names are shown in Fig. 12.2.

The division of the forebrain into two starts early, but progresses slowly. Even before it has properly started, the formation of the eyes begins. They are developed from the region which will eventually form part of the second forebrain vesicle (the diencephalon), and are originally merely exaggerated lateral swellings of the brain tube. These bulge out through

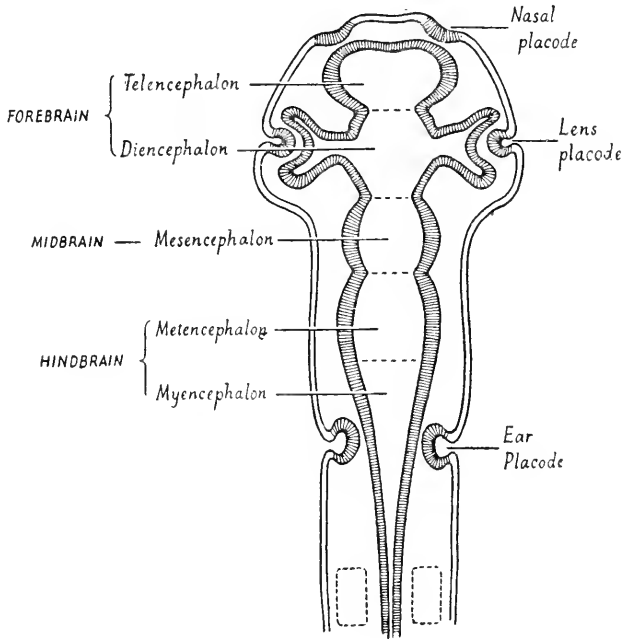


FIGURE 12.2

Diagram of the various regions of the brain, the eye-cups, and the nasal, lens and ear placodes.

the mesenchyme which lies between the brain and the ectoderm (Fig. 1.1, p. 7), and come up against the latter. At this stage, two things happen. The first is the formation of the 'optic cup'; the swelling begins to be transformed into a roughly mushroom-shaped structure, with a rather narrow stalk leading up to the brain while at the other end the part which comes into contact with the ectoderm is folded inwards rather as one pushes in the foot of a sock before putting it on. The cavity thus formed is the eye-cup, and is approximately circular except for a groove, the choroid fissure, on the ventral side. The layer of tissue which lines the cavity of the cup becomes the light-sensitive retina, and the outer layer or

tapetum develops into part of the pigmented and protective coverings of the eye.

The second ingredient in the developing eye is the lens. This originates from an infolding of ectoderm at the point where the optic swelling touches it. Its inception normally depends on a process of induction. Some time before Spemann discovered that the appearance of the main axis of the embryo depends on induction by the primary organisation centre, he had found that the eye-cups of newt embryos, transplanted so as to come in contact with the ectoderm of the belly, caused the appearance there of an induced lens (Reviews: Mangold 1931, Spemann 1938, Needham 1942).

The experiment reveals a classical example of a *secondary* organiser, that is, one which is effective after the primary organiser activity is completed. Very many of these operate during the period in which the earliest organ rudiments are appearing (Fig. 12.3). In fact it is probable that in the

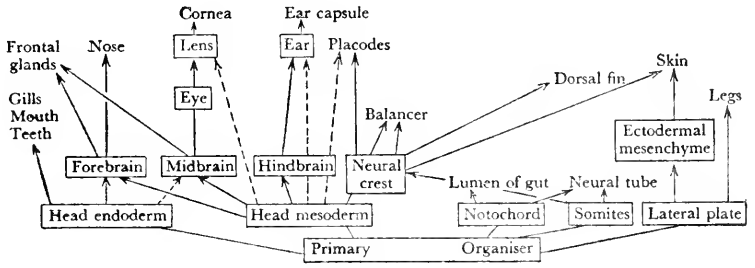


FIGURE 12.3

Diagram of secondary organisers in the newt.
(After Holtfreter.)

vertebrates every rudiment owes its origin to a secondary organiser or to a complex of organising influences; they are found in all the vertebrate classes which have been studied. For instance, lens induction by the eye-cup has been demonstrated by Waddington, van Deth and others in the chick (Review: Waddington 1952*a*). And to give further examples, two other organs which require notice at this point also depend on secondary organiser complexes. The nervous component of the nasal organ arises as two ectodermal thickenings or placodes near the anterior end of the forebrain; and it can be shown by transplantation experiments that the forebrain induces them. Again, the ears originate near the hindbrain from similar lens-like ectodermal thickenings, around which, after they have been folded inwards, the mesodermal structures are later formed. Experiments have shown that in this case the inducing system is complex; the

hindbrain plays an important part in calling forth the thickenings, but so does the local mesoderm, and a fully normal ear can only develop when both components induce together in a harmonious way.

There are in reality whole sequences of 'secondary' organisers, acting one after the other; perhaps they should be classed as secondary, tertiary, quaternary organisers and so on, but it would be difficult to do this in any very sensible way. For example, after the eye-cup has induced the lens, the whole complex then induces the skin lying above it to become transparent and to differentiate into the cornea, and the mesoderm which clothes it to become the sclerotic coats of the eyeball. Again the original ectodermal placode of the ear induces the mesoderm to form the other parts of the ear structure.

Within its ectodermal covering, the head of course contains not only the brain but also an infilling of mesoderm and some endoderm. The latter forms the pharynx, or anterior part of the gut, and will be discussed below in connection with the latter. It remains here to say something about the mesoderm. This tissue has a double origin. The greater part of it is formed by invagination through the blastopore or primitive streak, and this is the part to which we have so far paid most attention; but some mesoderm is also formed from cells which follow quite a different path. When the two neural folds finally come together and fuse to form the neural tube, some of the cells at the two fusing edges break loose and move down between the tube and the overlying ectoderm. These have been given a variety of names by various authors; sometimes they are alluded to as 'mesectoderm', a word which is also used for the epiblast of a blastoderm before the mesoderm has invaginated and thus become separated from the ectoderm; a somewhat better name is 'ecto-mesoderm', which is not so ambiguous, but probably it is simplest and best to speak of this second contribution to the mesoderm as the 'neural crest material', a phrase which clearly describes what is meant (Fig. 12.7, p. 266).

In most of the trunk, the neural crest material is scanty compared with the whole bulk of the mesoderm; it eventually forms the pigmented cells of the skin and contributes to the spinal ganglia. In the head it plays a much more important part, and forms large masses of tissue, which develop not only into some of the cranial nerves but also give rise to many parts of the cartilaginous skeleton (Hörstadius and Selman 1942, de Beer 1947).

3. *The gut: anterior portion*

In the Amphibia, it looks at first sight as though the formation of the gut is extremely simple, since a complete closed tube is formed during the

process of gastrulation (but see p. 261). At first the endoderm forms only a trough, which is covered dorsally by the mesodermal layer of which the notochord is a part, but fairly soon the edges of the trough grow round to meet under the notochord, so as to form a closed tube made wholly of endoderm. The anterior part of this tube reaches forward under some of the head structures which have just been described. At its tip, the tube is at first closed, and in this region there is no mesoderm lying between it and the ectoderm. In the mesoderm-free area, the gut first fuses with the ectoderm, and then both layers break down, so that an opening appears leading from the exterior into the lumen of the gut; this is the rudiment of the mouth.

Just posterior to this, the gut becomes swollen into a large cavity, which is known as the pharynx. The wall of this becomes thrown into a series of deep folds, which run from top to bottom on each side. These folds, five in number, eventually reach through the mesoderm to the ectoderm, and fuse with it. Again, the combined ectoderm and endoderm breaks down and forms an opening. In this case, the openings correspond to the gill openings of fish; in the higher groups of vertebrates they make only a transitory appearance during early embryonic life, before becoming transformed into something else; in some cases they never open completely at any stage. Their fate in the various groups is too complex to be followed in detail here. (For instance, the most anterior forms part of the Eustachian tube and the tympanic cavity of the middle ear.) Between each of the gill slits (which are also known as pharyngeal or visceral clefts) is a gill 'arch'. In these there is a core of mesoderm between the ectoderm and endoderm, and in the centre of this core, an important blood vessel, one of the so-called aortic arches, will eventually run.

Essentially the same structures are formed in the chick, but by somewhat different processes, since the anterior part of the gut, as we have seen (p. 252) appears not during gastrulation but in connection with the head fold. The formation of the mouth and pharynx, however, involves the same processes of the local fusion of ectoderm and endoderm, followed by their breaking down to give place to an opening.

In the chick, another derivative of the pharynx makes its appearance at a fairly early stage. A pocket of endoderm pushes out from the floor towards the posterior end of the swollen pharyngeal region, and rapidly elongates and extends backwards; it soon branches into two. This is the rudiment of the trachea leading to the two lungs. In the Amphibia it arises in the same region and in a similar way, but at a considerably later stage.

4. *The trunk: ectodermal organs*

As might be expected, it is in the main body of the embryo that the three fundamental layers, of ectoderm, mesoderm and endoderm, are most typically developed and most clearly defined.

The ectoderm produces only three main organs or organ-systems. The mid-dorsal part forms the central nervous system, which in the trunk is in the shape of a tube with thick walls, a thin floor and roof, and a cavity which is high and narrow in transverse section. From this tube the ventral motor roots grow out in a series corresponding to the somites. The dorsal sensory roots, with the accompanying ganglia, although they are eventually so closely associated with the main central nervous system, have a different origin in that they arise from the neural crest material. This, the second of the three ectodermal systems, also comprises the sympathetic nervous system and contributes to the mesodermal sheaths of the spinal cord; moreover the greater part of the pigmented cells of the body, whether they lie in the skin or in the linings of the gut and other internal organs, arise from the same source (Rawles 1948, 1953) (the most important pigmented tissues with another origin are those of the iris and outer layers of the eyeball). Finally the third system formed from the ectoderm is the outer layer of the skin, the epidermis. The skin as a whole is a composite structure, a major part of its thickness being contributed by the mesodermal layer known as the dermis, which originates from the upper layers of the somites.

The skins of different classes of vertebrates are by no means simple structures, but contain several sorts of sweat glands, oil glands and so on. Two of these structures are of particular importance, and are worth mentioning very briefly, namely feathers and hairs. Feathers are formed from a so-called feather germ or follicle. This is a slight hillock on the skin, from the tip of which a canal extends down through the thickness of the follicle. At the base of the canal lies a conical papilla, and it is from this that the feather actually grows. The papilla is a double structure, with an epidermal (ectodermal) cap fitting over a dermal (mesodermal) core. Many experiments have been performed on this structure, since it is one of the few organs in a bird which will continue to go through its developmental performance late into adult life; when a feather is plucked it will be regrown from its original follicle. Wang (1943) was able to peel the epidermal cap away from the core, and transplant the latter into follicles from which their own papillae had been removed. He showed that the dermal core can induce such a follicle to produce a new epidermal cap, and eventually a feather. This is an example of a 'secondary' organiser

acting at a very late stage. The induction is not effective on non-follicular ectoderm; and it is very remarkable that when a feather is induced in this way, the details of its structure (for instance, whether it is typical of the breast or the back) is determined not by the dermal core which induced it but by the place of origin of the follicular epidermis which responded to the inducing stimulus. (For a longer discussion of feather formation see Waddington 1952*a*).

Hairs also are formed from follicles which possess both ectodermal and mesodermal components. Much less is known about their structure and the inducing actions, if any, which go on within them; but since one special type of hair, namely wool, is one of the major raw materials of industry, a great deal is known about its development in other respects. Perhaps the aspect which is of most interest to general embryology is the study of the various shapes which may be taken by wool fibres, a subject which has been largely opened up through the pioneer investigations of Dry (1933–34). There are many different types of wool fleeces; each of them is characterised by a particular array of fibre-types, which occur with particular frequencies and can be distinguished by their length, thickness and the sequence of curves along them. It has long been known that the follicles occur in groups in the skin; within each group, the follicles develop in series, first the central primary, then the lateral primaries, finally one or two waves of secondaries. Fraser (1952) has recently presented evidence that each type of fibre is formed from some particular type of follicle; and he has elaborated a theory which shows how one could account for the shapes of the fibres, which differ in the series of curvatures along their length, by the interaction between a varying growth rate and a regular periodic chance in the direction in which the fibre is pushed out. If all the fibres grew at a constant rate, they would all have a regular wavy form. But Fraser suggests that actually they grow at a rate which is dependent on the efficiency of their follicle in competing with other follicles for a limited quantity of available substrate; and this efficiency is supposed to change according to the time of origin of the follicle. In the various breeds of sheep there are differences, not only in the relative numbers of the primary and secondary follicles, and in their density per unit area, but also in the curve which relates the efficiency to the time of origin of follicle (Fig. 12.4). In this way, the different shapes of the final structures can be accounted for in terms of physiological processes. The theory, although still in need of further testing, is a good example of the kind of mechanism one has to search for in the attempt to explain the facts of structure in terms of functional activities.

The formation of hairs, which usually grow very rapidly in comparison

with the cells nearby, would seem to provide a good opportunity for studying the processes of protein synthesis in embryos. Some histochemical work on the growth of wool has been published by Hardy (1952), who finds that, as usual, RNA is present in high concentration in the cells in which growth is occurring most rapidly. The distribution of some other substances thought to be important for protein synthesis, such as

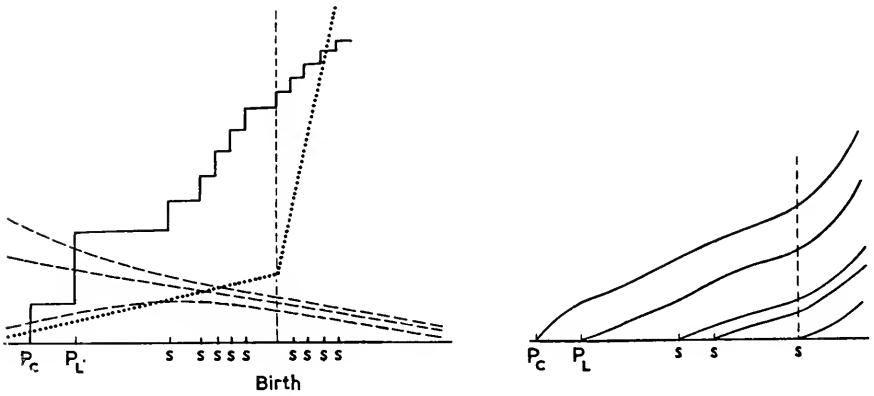


FIGURE 12.4

The rate of growth of the wool fibre from a follicle depends on (1) the amount of available substrate, which is proportional to the area of skin, (2) the efficiency of the follicle divided by the sum of the efficiencies of all the other follicles with which it is competing. In the figure on the left, the time of initiation of the primary (*Pc* and *Pl*), and secondary (*S*) follicles is shown below. The dotted line shows the increase in skin area. The group of dashed lines show various relationships between the efficiency of a follicle and its time of initiation—the upper curve being that characteristic of a coarse fleece, the lower one of a fine fleece. The stepped line shows the increase in the total efficiencies of all the follicles. The figure on the right shows the growth curves of a series of fibres calculated on this basis (medium fleece). (After Fraser 1952.)

sulphydryl-containing proteins, is also interesting, but the whole range of data cannot yet be fully interpreted (Fig. 12.5). Investigations by Lees and Picken on the influence of genetic factors on the rate of synthesis and the kind of protein produced in *Drosophila* hairs are referred to on p. 337.

5. *The trunk: endodermal structures*

It is, of course, the endoderm which forms the innermost structures, namely the gut and its annexes. In fact it produces only the central core of these, since the adult organs include an investment of mesoderm which clothes the original endodermal rudiments.

We have seen that in the Amphibia the process of gastrulation itself

produces a tubular primitive gut reaching from the blastopore, which occupies the position of the anus, to the head. It was for long believed that the cavity of this remained as the lumen of the adult gut, and that the various organs associated with the gut developed as outpocketings from its walls. The most striking of these pockets develops just posteriorly to the pharynx, and is usually considered to be the rudiment of the liver. According to recent studies by Balinsky (1947), however, this conventional interpretation is in error. He claims to have shown, by vital staining

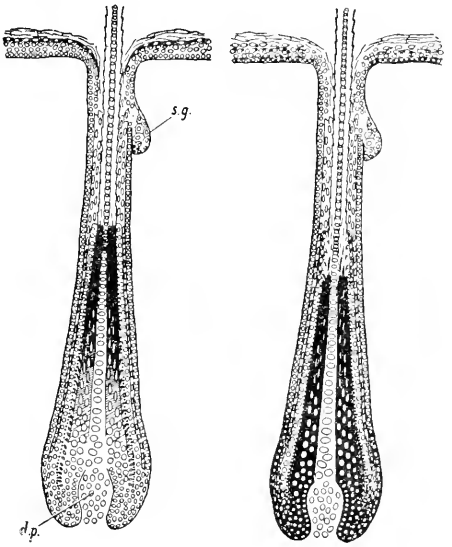


FIGURE 12.5
Semi-diagrammatic longitudinal sections of hair follicles in the mouse. On the left, stained to show sulphydryl groups, on the right to show cytoplasmic RNA. *S.G.*, sebaceous gland; *D.P.*, dermal papilla. (From Hardy 1952.)

experiments, that the so-called liver pocket continues to elongate backwards until it eventually reaches right back to the blastopore region. In fact, it constitutes the whole of the definitive gut, and outfoldings which will give rise to the liver, the pancreas and other organs appear in its walls, not in those of the primitive gut, which eventually disappears completely. These conclusions relate to Urodela. Nakamura and Tahara (1953) find that in Anura the situation is more like the conventional picture.

In the chick, the main stretch of intestine (the midgut) is formed by the continued backward extension of the pocket of the foregut. A similar

pocket, to be mentioned later, is formed under the tail, originating at a later stage than the foregut. It slowly extends forwards, and the two gradually come together near the middle of the body, leaving a narrow connection through which the gut opens out on to the underlying yolk; this is the umbilical cord. It is not till near the end of incubation that the chick gut becomes completely closed, and this is achieved, not by closing off the umbilical cord, but by the small remaining piece of yolk being drawn in inside the body, when the ectoderm and mesoderm close the hole through which it has entered.

6. *The trunk: mesodermal structures*

The mesoderm makes up the greater part of the bulk of the adult body, between the outer ectodermal layer of the skin (the epidermis) and the inner endodermal layer of the gut (the intestinal lining).

The first organs which become separated out from the rest of the mesoderm are those lying along the dorsal side. Immediately under the centre of the neural tube, a long rod is formed; this is the notochord, which acts as the first element in the skeleton, providing a longitudinal stiffening of the axis of the body. Its stiffness is not due in any large part to the production of hard or inflexible substance, but to the fact that the cells become swollen with fluid, so that the whole rod becomes turgid and stiff. The principle is one which Nature has made use of in other cases, where a temporary stiffness is required for physiological functioning. In this case the turgor is more permanent since the notochord induces the neighbouring mesoderm to secrete around it a thin but inelastic sheath (Mookerjee 1953).

On each side, the layer of mesoderm is thicker at its median edges, where it abuts on to the notochord. Very soon, this thicker portion becomes more or less separated from the more lateral parts by the formation of a thinner longitudinal strip; this is known as the intermediate mesoderm, and from it the kidneys will develop. The thicker medial strips of mesoderm soon become cut up by a series of transverse grooves into separate blocks, known as the somites, which lie in pairs on each side of the notochord. The transverse grooving, and thus the appearance of the distinguishable somites, begins at the anterior end, and gradually progresses posteriorly, until there may be forty or more pairs of somites; the number differs in different species. One or more of the most anterior pairs may break up and disappear fairly shortly after their formation. The remainder of the somites persist and gradually give rise to the main segmental organs of the trunk, particularly the vertebrae and the associated segmental muscles; they also contribute to the dermal layer of the

skin. Somewhat unexpectedly at first sight, the boundaries between the vertebrae do not correspond to the grooves between the somites; in each vertebra of the adult the posterior half has been produced by the anterior part of one somite while the anterior half is derived from the posterior part of the somite next forwards in the series. This arrangement ensures that the muscles arising within each somite are from the beginning joined to two contiguous vertebrae.

In *Amphioxus*, and in the primitive vertebrates such as cartilaginous fishes, the somites are hollow (Fig. 12.7). The cavity within them (the myocoel) is part of the general body-cavity, and is continuous with the larger and better-developed space (the splanchnocoel) which forms within the lateral mesoderm. Both cavities together are known as the coelome. In higher vertebrates the myocoels are small and not always easy to detect. It is within the lateral parts of the mesoderm that the main body-cavity of the adult develops; the mesoderm lying above the space becomes closely applied to the ectoderm, and forms the dermal layer of the skin, while that below lies against the endoderm and produces the muscular layer of the gut. The connection between the upper and lower layers of mesoderm persists in certain places, and provides the mesenteries by which the gut and its derivatives are attached to the main part of the body.

7. *The tail and hind part of the body*

At the stage when, in the amphibian, the neural plate becomes clearly delimited and the neural folds appear, the greater part of the plate is destined to form the brain and the nervous system of the anterior region of the body. The material for the whole posterior part of the trunk, and for the tail, is concentrated in a small region near the remains of the blastopore. In the chick, the material for the brain and anterior end arrives in place, and begins to differentiate, still more in advance of that destined to build the posterior end; and by the time only five or six pairs of somites have appeared, the primitive streak has already become quite short, although the greater part of the trunk is still to be produced. These facts have suggested to some authors, of whom the most authoritative in recent years was Holmdahl (1939), that the gastrulation process as we normally conceive it is responsible only for the formation of the anterior part of the animal and that the posterior part is produced by some radically different process which goes on within the small remnant of blastopore or primitive streak. This region, from which the posterior part forms, is known as the tail-bud. The authors who argue that the processes going on within it are quite different from those of gastrulation nevertheless

do not agree on the position of the dividing line between the anterior part and the posterior part for which it is responsible.

The existence of this theory has led to an intense study of the development of the region in the neighbourhood of the blastopore of the neural-plate stage amphibian. Bijtel (1931) first showed that invagination is still proceeding at the blastopore even after the appearance of the neural folds; and further that some of the material between the posterior ends of the folds (i.e. material of the neural plate itself) will actually form mesoderm (Fig. 9.10, p. 166). More recent authors, particularly Pasteels (1939), Nakamura (1942) and Chuang (1947) fully confirm this, and demonstrate conclusively that the processes going on in the late blastopore are essentially invagination processes broadly similar to those of gastrulation proper. The main differences are two. The first is relatively trivial. Throughout the earlier phases of gastrulation, the dorsal midline above the blastopore is occupied by presumptive notochord. In the neurula stage, however, the last piece of presumptive notochord invaginates before all the somite mesoderm has moved into the interior; and in its final stages, therefore, the dorsal lip of the blastopore consists of presumptive somite material. The second difference is perhaps more important. Combined with the normal in-rolling movement of gastrulation, there is in the late blastopore a stretching by which the tail is thrust out as an elongated structure. This produces a rather complicated system of movements, but it does not alter the essential fact that each region of tissue moves in a precisely defined way and reaches a definite final position. There is no reason to believe, as we are urged to do by Holmdahl, that the tail-bud is a mass of indifferent tissue from the general undifferentiated bulk of which the posterior neural tube, somites and chorda appear.

In the chick, the precise movements occurring in the late primitive streak are not so fully known, but there seems no reason to doubt that here again the posterior part of the body is produced by gastrulation processes essentially similar to those which give rise to the anterior end (cf. Pasteels 1939, Waddington 1952*a*).

Associated with the tail-bud is the hind end of the gut. This opens to the exterior through the anus, or cloaca, which is formed near the site of the blastopore, but not directly out of it. In forms such as Amphibia, which during gastrulation possess an open blastopore leading in to the cavity of the gut, this opening is closed by the fusion of its lips as invagination terminates. From the hind end of the gut, two pockets are then pushed out. One extends from the ventral side of the gut, and this reaches the ectoderm (which may fold inwards to meet it); the endoderm and ectoderm first fuse, and then break down to give an opening which becomes

the definitive cloaca and anus. The other endodermal pocket starts from the dorsal side of the gut and extends backwards into the tail, where it is known as the tailgut. The extent to which it is developed varies greatly in different species even of the same group of animals; thus in the frog it is inconspicuous, while in some toads it forms a rather long tube, which extends right round the posterior tip of the notochord, its lumen becoming confluent with the cavity of the neural tube (the junction being known as the neurenteric canal) (Fig. 12.6).

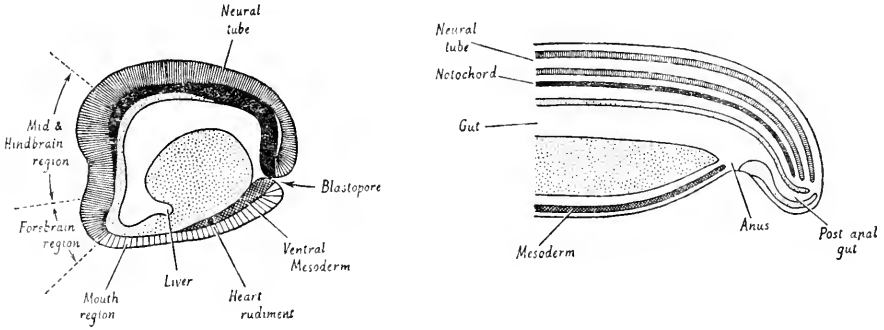


FIGURE 12.6

On the left, a diagrammatic longitudinal section through a newt neurula. Closely lined neural plate, showing the future regions into which it will develop; darkly dotted, notochord; lightly dotted, endoderm; cross-hatched, ventral mesoderm. On the right, section through the tail region at a later stage.

8. *The kidneys*

There are three main types of 'kidney' developed in vertebrate embryos. First, the pronephros forms in a more or less anterior position; later the mesonephros appears further posteriorly; and finally the metanephros differentiates still further back. All these organs are paired, one appearing on each side of the body in the so-called 'intermediate' mesoderm, i.e. that lying between the somites and the lateral plate (Fig. 12.7). The basic unit, from which the kidneys are constructed can be thought of as a tube, one end of which opens into the coelome, while the other is connected with a duct which leads the secretion away towards the posterior; and somewhere between these two ends, the tube becomes closely apposed to a blood vessel. In functional kidneys, this simple unit becomes highly modified and complicated; we shall not discuss these details which belong rather to the province of comparative morphology than that of general embryology. But it is necessary to say something about the duct.

This is a tube which leads, in the early stages, from the pronephros to the posterior where it opens into the cloaca. It is at that time known as the pronephric duct. Later the mesonephric tubules become connected with it; and in the higher vertebrates the pronephros degenerates and disappears; the duct is then entitled to be called the mesonephric duct. (It is also referred to as the Wolffian duct.) Finally, a diverticulum is pushed out from it, starting from the region near the cloaca; this makes contact with

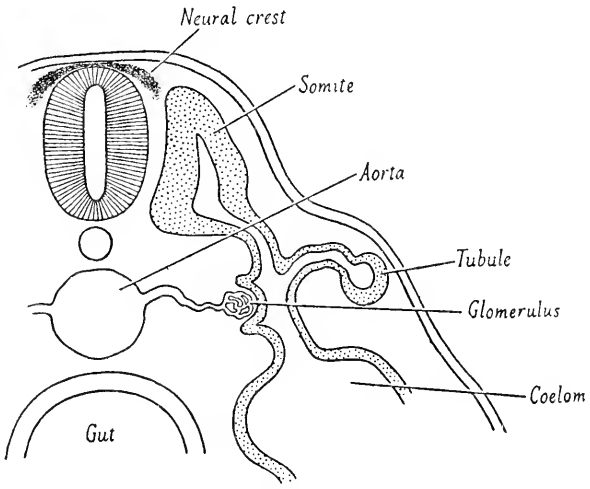


FIGURE 12.7

Diagrammatic section through the anterior trunk of a vertebrate, showing the relations of the mesonephric tubules.

the metanephros, and is known as the metanephric duct (Fig. 12.8). Closely associated with it is another duct, the Mullerian duct; this leads from the exterior to the gonads, which originate near the kidneys, though they may later shift into another region of the abdomen.

The comparative study of kidney formation in the different classes o. vertebrates, besides being of great interest from the anatomical point of view, illustrates one or two points of importance for general embryology.

In the first place, we may note that the pronephros, which is an actively functional excretory organ in amphibian tadpoles, is formed opposite a well-defined group of somites; numbers 2, 3 and 4 in Anura, and numbers 3 and 4 in urodeles (cf. Cambar 1949). In the chick, in which the pronephric tubules are rudimentary, and never function as excretory organs, they are developed transitorily opposite somites 5 to 16. This is a good example of an organ being moved along the length of the body

during evolution, a phenomenon which is of fairly widespread occurrence.

The epigenetics of kidney development has been rather extensively studied; recent reviews are those of Fraser (1950) for the vertebrates in general, Cambar (1948) for Amphibia and Waddington (1952*a*) for birds.

In the Amphibia, the capacity to develop into pronephros appears at a certain level of a gradient which runs from a high point in the chorda to a low in the lateral plate. This was demonstrated by Yamada (1940), who showed that if lateral mesoderm is taken from a position in the neurula some distance away from the embryonic axis and cultivated in isolation, it will develop only into tissues normally formed from such lateral regions, such as blood, while if chorda is added to the isolate, pronephric tubules appear (see Fig. 10.10, p. 191). It is to be presumed that the first step in the development of the more posterior intermediate mesoderm (which will form mesonephros) is taken in the same way; and it seems not unlikely that a similar process occurs in the bird embryo, though this has not been definitely proved (cf. Waddington 1952*a*).

There is no evidence that the amphibian pronephros requires any further stimulus, after its position on the medio-lateral gradient is fixed, before being able to complete its development. For the more posterior kidneys (mesonephros and metanephros), however, something further is necessary, namely an inductive influence which is normally exerted by the pronephric duct. As we have seen, this duct grows backwards from the region of the pronephros. If its backward extension is prevented (e.g. by a transverse cut which fails to heal completely) no sign of the duct appears in the posterior region, and only minor traces of the mesonephros develop. The dependence of the amphibian mesonephros on an inductive stimulus from the pronephric duct was first suggested by Miura (1930) and later work by many authors has fully confirmed it (O'Connor 1939, Cambar 1948). In birds, Boyden (1927), Grünwald (1937) and Waddington (1938) have found a similar situation (Fig. 12.8).

In birds the pronephros never functions as an excretory organ, and Waddington suggested that it had been retained in the ontogeny of the animal simply because it is an essential step in the formation of the pronephric duct which is itself necessary as the inducer of the mesonephros. Cambar (1948) has criticised this suggestion on the grounds that in the Amphibia the pronephric duct arises from a mass of tissue which is distinct from, although it lies immediately in contact with, that which gives rise to the pronephros itself; moreover he states that the growth of the duct is independent of the continued presence of the pronephros, whence

he concludes that that organ is quite unnecessary for the production of the mesonephros. However, it is not at all clear that even in the Amphibia the original production of the rudiment of the duct is quite independent of the presence of the pronephros, and in birds the two components are so intimately associated that it is difficult to imagine the duct being produced without at least some transitory appearance of the tubules of the

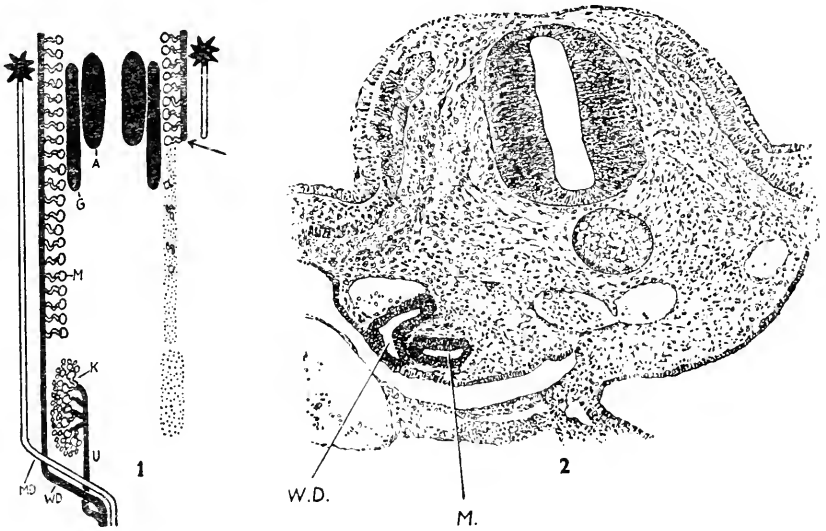


FIGURE 12.8

Figure *1* shows, on the left a diagram of the normal urogenital system, comprising the adrenal (*A*), gonad (*G*), mesonephros (*M*), Mullerian duct (*MD*), Wolffian (or mesonephric) duct (*WD*), ureter (*U*) and definitive kidney (metanephros) (*K*). The parts which develop independently of others are shown in solid black, those resulting from inductive actions in outline. On the right is the result of preventing the mesonephric duct from growing posteriorly beyond the arrow; the posterior mesonephros and other organs fail to appear. (From Gruenwald 1952.) Figure *2* is a section through a chick embryo in which the posterior growth of the pronephric duct was prevented on the right side. There is not only no sign of the duct (*WD*) but the mesonephros (*M*) has also failed. (From Waddington 1952*a*.)

nephros. There seems, therefore, no good reason to doubt that we have, in the appearance of the pronephros in birds, an example of the retention during evolution of an organ for the sake of its function as a component of the epigenetic system, rather than for any contribution it makes to the physiological functioning of the embryo as a metabolising organism.

Although one may probably conclude that the pronephric duct is an important factor in the epigenetic system, it is by no means the only actor on the stage. The competence of the intermediate mesoderm plays

an important part. Even in the absence of the duct, accumulations of what one may consider as 'pre-mesonephric' cells may put in a transitory appearance. Further, the inductive influence of the duct is only effective if it operates on intermediate mesoderm (which has probably been brought to a state of competence by its position in the medio-lateral gradient); and this mesoderm can, in the chick at least (Grünwald 1942, 1943), react successfully to abnormal inductors, such as transplanted pieces of neural tissue.

Grünwald also showed that an inductive reaction is concerned in the production of the metanephros of the chick. Here the inductor is the diverticulum which pushes out from the region where the pronephric duct joins the cloaca. He discovered the remarkable fact that if a piece of the main (mesonephric) region of the duct is substituted for this diverticulum, and allowed to act on the presumptive metanephric tissue, it succeeds in inducing kidney, but mesonephros rather than metanephros. This is one of the comparatively few cases in which the character of an induced organ is determined by the nature of the inducer rather than by the competence of the reactant.

Grobstein (1953*a*, *b*) is analysing the inductive reaction between the duct diverticulum (uretic bud) and the metanephric mesoderm in the mouse, which is probably rather similar. He finds that the mesoderm can be induced to form tubules by a variety of different types of tissue, all of which are epithelial in character and to that extent at least similar to the uretic bud which is the normal inducer. An important result is that the inducing agent given off by embryonic spinal cord, for example, can pass through a 20μ thickness of an artificial porous membrane; this is one of the most direct proofs that induction may be carried out by diffusible chemical substances. Grobstein has used the same methods for investigating other examples of the development of glands which contain both epithelial and mesenchymal components, particularly the submandibular (salivary) gland. By careful exposure to trypsin solutions, he can separate the epithelial and mesenchymal tissues; the epithelium can then be cultured in combination with mesenchyme from its own type of gland or with that from some other organ. He finds that the epithelium differentiates typically only when combined with its own type of mesenchyme; other mesenchymal tissues may partially inhibit the spreading tendency which is usually seen in isolated epithelium, but do not induce the formation of normal tubules. If the epithelium and mesenchyme are separated by a fine-grain filter membrane, the inhibitory action of foreign mesenchyme passes through, and the specific effect of like mesenchyme also does so to some extent, though not completely; it induces the formation of tubules but not quite typical ones (Fig. 12.9).

The reproductive organs of vertebrates develop in rather close association with the kidneys and their ducts. We shall not deal with them here; recent summaries of the literature may be found in Nelsen (1953) and Nieuwkoop (1946). The germ-cells themselves originate at considerable distances from the glands in which they eventually lie, and reach them after performing a peculiar migration, as isolated mobile cells, through the intervening tissues of the embryo. The place of their origin seems to be surprisingly different in different groups; probably the lateral

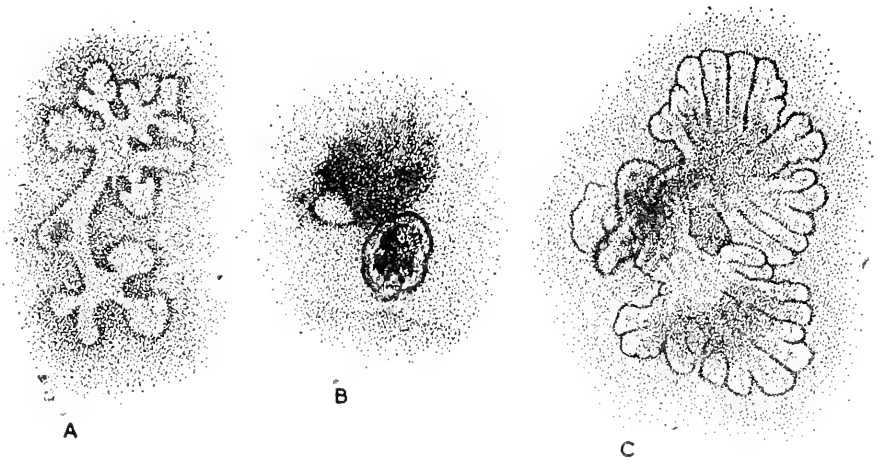


FIGURE 12.9

A shows the epithelium of the submandibular gland of the mouse growing in tissue culture combined with mesoderm from the same source: the epithelium is forming typical tubules. In *B* the epithelium is lying on top of a porous membrane, below which is mesoderm from another source (maxillary region): no morphogenesis. *C* is a similar culture but the mesoderm below the membrane is from the submandibular gland: tubule formation has been induced, although the morphogenesis is somewhat abnormal. (After Grobstein 1953.)

mesoderm in urodeles, the posterior dorsal endoderm in Anura, endoderm lying anterior to the head in birds, yolk-sac endoderm and mesoderm from the posterior end of the primitive streak in mice. An introduction to the literature can be found in Nieuwkoop (1949), Willier (1950) and Chiquoine (1954).

9. *The limbs*

The limbs first appear as slight external swellings which fairly rapidly elongate. In their early stages they consist of condensations of mesoderm

covered by epidermis which does not differ from that of the rest of the body. The mesoderm cells at first show little sign of particular differentiation, but form a rather loosely aggregated mass of mesenchyme. As the limb elongates, the cells towards the centre of it become more tightly packed, forming a number of condensations within the mesenchyme (Fig. 20.5, p. 429). These group themselves into the pattern of the skeletal elements of the normal limb, and gradually differentiate, first into cartilage and then into bone. Meanwhile the remaining mesenchyme develops into the muscles.

The development of the limbs has been very extensively studied, and provides examples of a number of points which cannot be so well illustrated in any other field.

At about the time of the First World War, Ross Harrison (1918) began a long study on the polarity and asymmetry of the limbs of urodeles. The subject was also pursued by a number of his students, such as Detwiler and Swett. The most recent summary of the extensive literature of this group of workers is that of Swett (1937) and the main contribution since then is an extensive and important work by Takaya (1941).

It is clear that a fully developed limb must be either a right or a left limb and that these two have essentially different asymmetry, being, in fact, mirror images of one another. The genesis of this asymmetry can be studied by excising the presumptive limb region from an embryo and grafting it back in such a way as to change the relation between the polarity of the graft and that of the host body. Consider, for instance, the forelimb of a newt. In the tail-bud stage the region from which this limb will develop is represented by a circular area on the side of the body just below somites 3 and 4. Suppose that the limb area on the left side of a newt embryo was cut out, then pushed up to the dorsal midline and down the other side, and eventually grafted in place of the right limb area, which had been previously removed. Then it is clear that its anterior end would still point towards the anterior end of the whole body, and its exterior side would still lie towards the exterior, but its dorsal side would now be below and its original ventral side uppermost. Such an orientation is described by saying that we have reversed the dorso-ventral axis of the graft but left its antero-posterior and medio-lateral axes unchanged. We could, of course, reverse both the antero-posterior and dorso-ventral axes leaving the medio-lateral one unchanged, by making a circular cut around the limb-forming area on one side and then rotating the area through 180 degrees about an axis perpendicular to its surface before allowing it to heal in again. By a variety of such methods one can, in fact, reverse at will any particular axis or combination of axes. After such

operations one allows the limb to develop and examines whether a disc whose antero-posterior axis was reversed actually develops back to front, in which case the axis may be said to have been already determined, or whether influences from the host's body succeed in causing a reversal of polarity within the limb-disc (Fig. 12.10).

It was soon discovered that the various axes are determined at different times. The antero-posterior one always becomes fixed first. According to Detwiler it is already determined by the middle gastrula stage, but Takaya gives reasons for doubting this, and suggests that the determination does not actually occur until the neural plate stage. Even so, this is much earlier than the determination of the dorso-ventral axis, which does

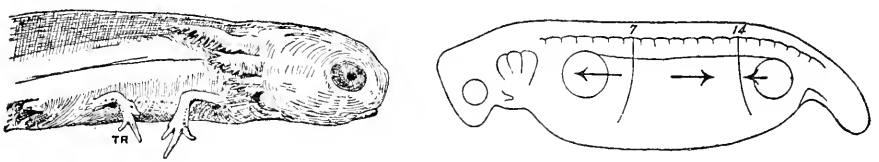


FIGURE 12.10

On the left, an axolotl embryo into which a limb-bud has been grafted with its dorso-ventral and anterior-posterior axes reversed. The transplant (*Tr*) has developed into a limb with reversed anterior-posterior axis but normal dorso-ventral axis. (After Harrison.)
On the right, a newt embryo showing the direction of the anterior-posterior field in different regions of the flank. The circles mark the normal positions of the limb-buds. (From Takaya 1941.)

not occur till the fairly late tail-bud stage, by which time the limb-buds have already become slightly elevated from the side of the body. It is shortly after this that the medio-lateral axis of the mesodermal component of the limb-bud becomes also determined.

The determination is certainly brought about by the tissues in the immediate neighbourhood of the disc. This can be shown by rotating a fairly large area in the limb region and then grafting into this region a limb-disc, with one or more of its axes reversed. In such cases it is the orientation of the immediately surrounding area rather than that of the whole host embryo which is decisive over the future development of the limb.

The nature of the influence which fixes the polarity of the limb-disc is still imperfectly understood. It clearly falls into the general category of what have been referred to as 'field characters', but that terminology does not tell us much about what it actually is. Harrison (1936) thought that the polarity might depend on some fine-grain structure of the tissue of

almost molecular dimensions and perhaps comparable to the oriented arrangements found in liquid crystals, but x-ray microscopy was not able to detect any such structure (Harrison, Anthony and Rudall 1940). Takaya, on the other hand, suggests that the fundamental factor is capacity for growth, which is highest in the antero-dorsal part of the early limb-disc. In his view it is the fixing of gradients in growth capacity which determines the polarity which the limb will exhibit.

If an early limb-disc is excised and grafted into a new position in such a way that its polarity is opposed to that of the immediately surrounding area, a frequent result is the development of a pair of duplicated limbs. These are nearly always mirror images of one another, one having the symmetry of a right limb and the other that of a left. The frequency with which this mirror-imaging occurs suggests that two limbs developing close to one another influence each other's polarity. This is confirmed by the results of grafting two limb-buds into each other's neighbourhood. It is found that even if they are grafted so that their polarities are concordant, nevertheless the two limbs which form often turn out to be mirror images, one of the two having had its original polarity reversed by its neighbour (Fig. 12.11).

It is a remarkable fact that the polarity of a normal embryo does not run in a constant direction throughout the whole flank of the animal. Opposite the anterior somites, from somite 1 to about somite 7, the polarity is such that it tends to cause the pre-axial side of the limb to develop on the side nearest the head of the embryo. The same is true of posterior regions where the hindlimb forms, from about somite 14 backwards, but in the midflank region, from the level of somite 7 to that of somite 14, the polarity of the flank is in the opposite direction (Takaya 1941) (see Fig. 12.10).

This reversal of polarity in the flank is clearly expressed in the experiments which have been made on the induction of limbs. This was first successfully accomplished by Balinsky (1925). He found that if an ear vesicle is transplanted into the flank of a young tail-bud embryo it induces the formation of a supernumerary limb. Later work (Balinsky 1933) showed that the same effect could be produced even more regularly by the implantation of a nasal placode. The organs used in these grafts can not, of course, be the normal inducer of the limb. It is not at all clear what organ or tissue fulfils this function in normal development. Attempts to induce limbs by the implantation into the flank of the pronephros, which lies near the site of the normal forelimb, have so far been unsuccessful.

The induction of limbs by such 'foreign' tissues as the auditory and nasal vesicles, raises in an acute form the problem of the specificity of the

inducing stimulus. Do the inducers give off some specific substance which is essential for limb formation, or do they only activate potentialities already fully present in the flank? Needham (1942) argues that the former alternative is the more likely on general grounds and is the better guide to future experimental work. Balinsky (1937), on the other hand, suggests that the inductors owe their power to a rather ill-defined quality which he speaks of as a 'high physiological activity', and he believes that it is this which sets in motion the inherent capacity of the flank mesenchyme to develop into a limb. The question needs much further study; no serious attempt has yet been made to investigate the phenomenon at a biochemical level. It seems likely that it will turn out to be very similar to the situation with which we are confronted in the induction of the neural plate:

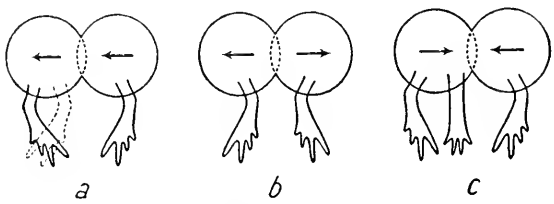


FIGURE 12.11

Mirror imaging when limb-buds develop near one another. In *a* the *ap* axes are concordant; one of the limbs frequently has its polarity reversed. In *b* the *ap* axes point away from each other, and both limbs retain their polarity. In *c* the *ap* axes point towards one another, and a supernumerary limb, of ill-defined polarity, often appears. (From Takaya 1941.)

that is to say, that the induction can be performed by relatively unspecific implants, but that these may act in a secondary way, their first effect being the release of specific substances within the reacting tissues. One single case has been described [Balinsky 1927] of the induction of a limb following the implantation of a fragment of celloidin into the flank. This may probably be compared with the induction of neural plate by treatments which produce 'sub-lethal cytolysis' (see p. 196).

Whatever the position as regards the specificity of the inducing stimulus, there is no doubt that the competence of the reacting material plays a large part in the production of the limb. One evidence of this is the asymmetry of the induced limb in the various regions of the flank. Back to the level of somite 7 the pre-axial side develops anteriorly, but between somites 7 and 14 the asymmetry is reversed (Takaya 1941). Again Balinsky (1933) showed that the frequency with which limbs are induced falls off fairly steadily from the region of the forelimb- to that of the hindlimb-bud

(Fig. 12.12). This gradient is probably not a straightforward reflection of the intrinsic capacities of the flank mesenchyme, but is partially a result

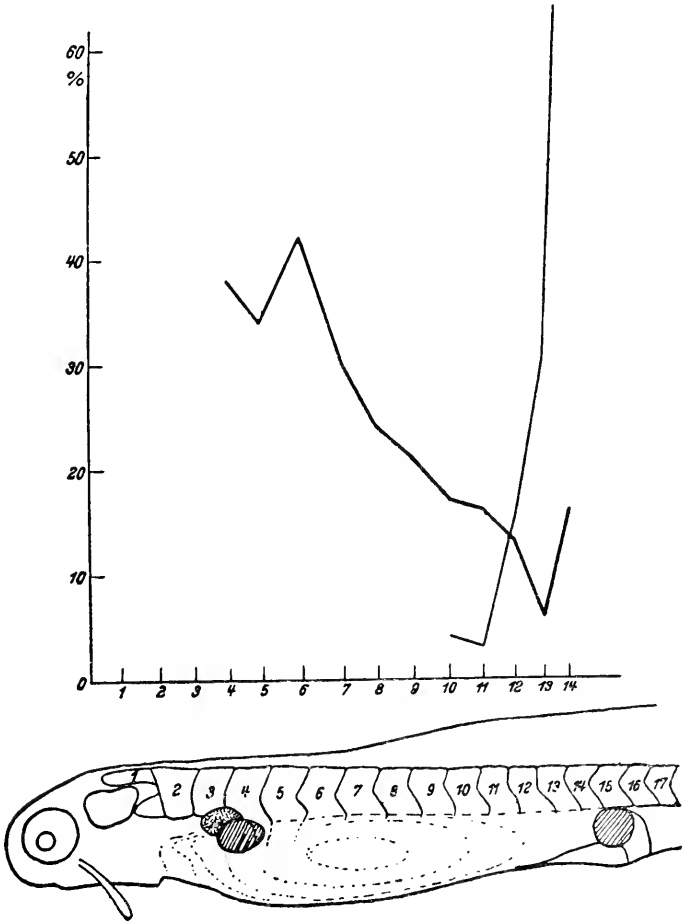


FIGURE 12.12

Limb induction in the newt. The graph above shows the frequency with which limbs (thick line) or pelvic girdles (thin line) were induced by the implantation of nasal placodes in the corresponding region of the flank indicated on the drawing below. In the drawing the position of the normal limbs is shaded, that of the pronephros dotted. (From Balinsky 1933.)

of the general influence of the body as a whole. Thus Takaya has shown that if material from the lowest point in the gradient (just in front of the hindlimb-bud) is transplanted to a more anterior position, and then submitted to the influence of an implanted inductor, the frequency of limb

induction is much higher than it would have been if the material had been left in its original location.

Another manifestation of the field of competence can be seen in the frequency with which the induction produces forelimbs or hindlimbs. Forelimb-like structures can be induced in the anterior region back to the posterior margin of somite 8, while hindlimbs can be induced in the posterior region, which extends forward to the anterior margin of segment 8. Thus there is a small region of overlap between the forelimb- and hindlimb-producing regions. Shoulder girdles are usually not induced, but the pelvis can be evoked very regularly in the posterior end of the field near somites 13 and 14. These three lines of evidence—the asymmetry of the induced limbs, the frequency of the induction and the character of the limbs induced—clearly demonstrate the existence of a field of competence in the flank, but do not show how far this is a property of the localised areas of tissue, or how far it is dependent on factors which act in a general way over the whole region.

In the period immediately after the limb-bud is formed, the various individual parts of it are still incompletely determined. A single bud may give rise to a duplicated pair of limbs, or, in other cases, two anterior half-buds may fuse to give a single limb. We shall not carry any further the discussion of the gradually increasing determination of the amphibian limb-bud (see Review of Mangold 1929), but instead turn to a consideration of the limb-buds in the chick, which illustrate certain other points of general significance.

In the chick, technical difficulties have made it impossible to investigate the symmetry relations of the early limb-discs as has been done in the Amphibia, nor is anything known about limb induction in birds. It is in connection with the later stages of development of the limbs that investigations on bird embryo have been most informative. The first point we shall notice is one which emerges from the investigations of Saunders (1948). He showed that the bulk of the material which forms the limb-bud at the earliest stage at which it can be recognised will eventually form the proximal parts of the limb. More distal parts are added later as the limb-bud elongates. During this process a specially important part is played by a thickened cap of ectoderm which forms the actual apex of the elongating bud. If this apical cap of ectoderm is removed, the laying down of further distal regions of the limb ceases, and the limb remains as a stump from which the distal parts are missing (Fig. 12.13). This does not occur in the Amphibia, where the proximal parts can form a complete limb; but in that group, of course, proximal stumps can regenerate their missing distal parts till quite a late stage, while such regeneration does not occur in the chick.

Young limb-buds of the chick can be excised from their normal site and planted into the coelom just lateral to the somites, where they will continue their development in a remarkably normal manner. In such situations the limb-buds are often very incompletely innervated. In the nerveless limbs the muscles atrophy nearly completely, and the growth rate is somewhat less than normal, but although no movement at the

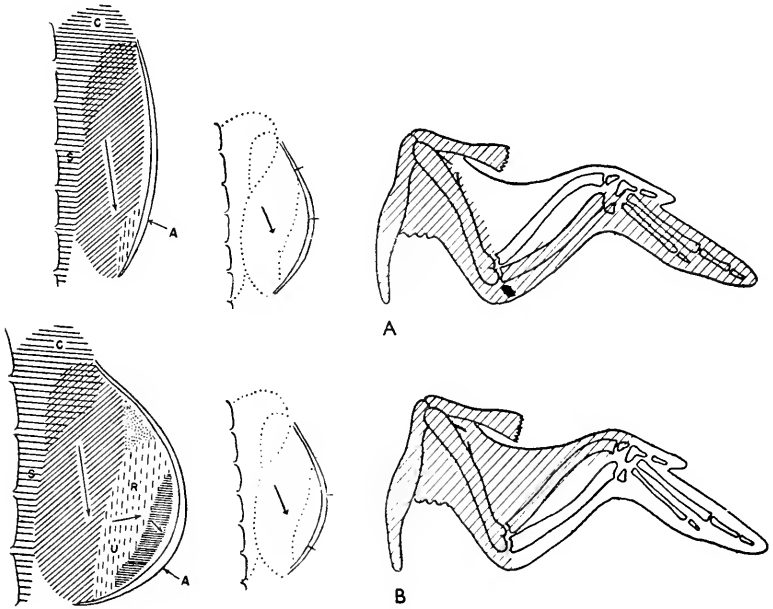


FIGURE 12.13

On the left, two stages (in section) of the limb-bud of the chick, showing the presumptive areas. *C*, coracoid; *S*, scapula; oblique shading, upper arm: dashes, forearm; horizontal shading, wrist and hand; *A*, apical ectoderm. On the right, the results of excising anterior (*A* above) or posterior (*B* below) regions of the apical ectoderm; the parts developing are shaded. (After Saunders 1948.)

joints occurs, the structure of the skeletal elements is exceedingly normal, even to the formation of the joint surfaces (Hamburger 1939) (Fig. 12.14). It is well known that if, owing to injury, a functional limb has to be moved in an abnormal manner, the joint surfaces may become modified in such a way as to facilitate this. It is clear, then, that function can play a part in moulding the structure of the skeleton, but the development of these isolated and functionless limbs shows that this structure can also develop with a considerable degree of perfection, solely under the influence of factors inherent within the developing tissues themselves. The interaction

of intrinsic and extrinsic factors in the architecture of the skeleton has been discussed particularly by Murray (1936). The development of limbs in abnormal sites in the body has provided the opportunity for a large number of investigations on the way in which the peripheral nerves make contact with the various parts of the limb, and also on the influence of an excess or deficiency of peripheral organs on the central nervous system. There is no space here to do more than mention this subject as one which

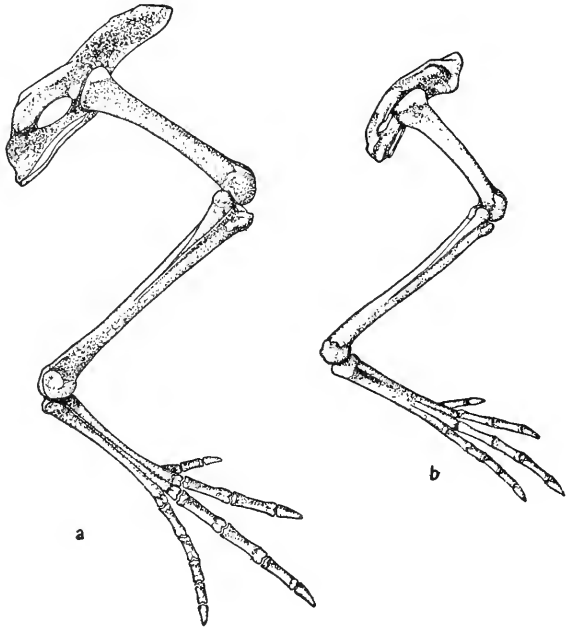


FIGURE 12.14

The development of a limb-bud of the chick, transplanted to the coelome and badly innervated (*b*), compared with that of a normal limb (*a*). (From Hamburger and Waugh 1940.)

provides evidence of epigenetic interactions between parts of the body continuing into the later stages of development. Recent reviews of the field will be found in such works as Piatt (1948), Weiss (1941, 1950*a*) and Detwiler (1936).

SUGGESTED READING

Classical papers are Harrison 1918, 1936, 1945; and Spemann's work on the eye, summarised 1938, pp. 40–97. For a general survey of experimental work on Amphibia, Needham 1942, pp. 290–309; on the chick, Waddington 1952*a*, pp. 140–200, and *Annals, New York Academy of Science* 1952, Volume 55, Article 2. Two very interesting lines of work, hardly touched on in the main text, will be found in Rawles 1948 and Landauer 1954.

CHAPTER XIII

GROWTH

1. *Overall growth*

In everyday usage the word 'growth' is used to mean any type of increase in size. This is obviously one of the important phenomena in embryonic development and requires discussion. There have been two ways of approaching the problem; one is content to accept the everyday meaning of the word and to study the increases which take place in whole embryos or in their parts; the other has attempted to start from some more precisely defined process of growth and to set up general norms from which the facts as they appear in the development of particular animals can be deduced as special consequences. This second attempt has not as yet proved very successful but it will be easier to exhibit the complexity of the whole situation if we start by discussing it (General Reviews: Needham 1931, Medawar 1945).

If we wish to consider a precisely defined process of growth we shall have to find some way of limiting the concept so that it is confined to the increase in size of something which retains a certain similarity to itself. Size may increase merely by the imbibition of water or by the laying down of relatively inert material such as shell, bone, cartilage, etc., and such processes obviously differ in kind from the increase in amount of the living material itself. Various definitions have been offered with the purpose of excluding them from the concept of growth as that is required for a precise theory. Gray (1931) speaks of growth as 'essentially concerned with the formation of new living material'. Medawar (1941) states that 'what results from biological growth is itself typically capable of growing'. Weiss (1949) gives a more formal definition; growth is 'the increase in that part of the molecular population of an organic system which is synthesised within that system', and he further amplifies this, pointing out that it means 'the multiplication of that part of the molecular population capable of further continued reproduction'. This puts its finger on the important point; if we are trying to formulate a precise concept of growth we must confine it to the increase in the amount of the system which is capable of growing. The main general problem which then requires study is this—at what rate does this increase take place and how does the rate change as time passes?

The simplest possible situation would be one in which the rate of multiplication per unit mass remained constant. We could formulate this

mathematically by the equation $\frac{1}{w}\frac{dw}{dt} = k$ constant (where w is the weight or mass of the system). This, of course, leads to the absolute size increasing ever faster and faster, at an exponential rate. The equation is solved to give

$$w = w_0 e^{kt} \text{ or } \log w = \log w_0 + kt$$

when w_0 is the initial size and k is a constant.

It is impossible to find a naturally occurring biological system which behaves so simply, and it is difficult to make one experimentally, though it can be done. If a small population of yeast cells, or bacteria, is inoculated into a large mass of nutrient medium, allowed to grow for a time, and a new inoculum transferred to fresh medium after a fairly short period, the growth rate per unit mass may be kept constant indefinitely. The essential points are that neither lack of nutrient nor the presence of harmful excreta are allowed to inhibit the system. If frequent transfers are not made, one or other or both these are certain to occur, and the rate of growth will slacken, till the growing mass becomes stationary and eventually begins to decline when the death-rate of cells overtakes the rate of increase. Samples taken from such declining cultures usually take a little time to get going when transferred to fresh medium, so that in the 'typical' growth curve of a colony of cells (yeast, bacteria, tissue-cultures and the like) the logarithm of size when plotted against time, is not a straight line, but has the form shown in Fig. 13.1.

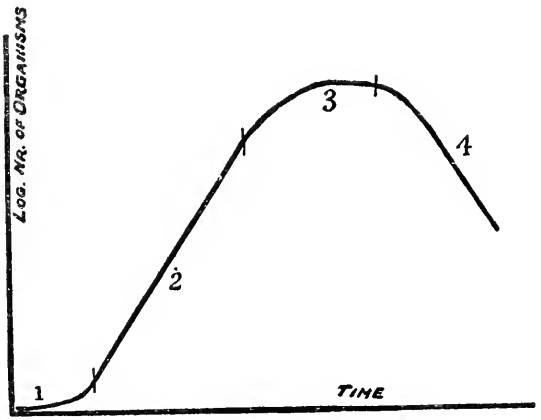


FIGURE 13.1

Typical growth curve of a population of isolated cells (e.g. yeast cells, bacteria, etc.). If the population starts with a group of cells taken from a non-growing colony, there is first a 'lag phase' (1) then a phase of exponential growth (2) in which the growth rate per unit mass is constant, then a phase of retardation (3) when the medium is becoming exhausted, and finally a regression phase (4) when the medium can no longer support the population.

The growth curve of a developing animal has a somewhat similar form; although there is no definite lag before growth commences, the curve relating mass to time is sigmoid or roughly S-shaped. It was Minot, at about the beginning of the century and following him Brody, who particularly pointed out that it would be more profitable to consider the so-called 'specific growth rate' (i.e. growth rate per unit mass $\frac{1}{w}\frac{dw}{dt}$, which is the same as $\frac{d \log w}{dt}$) rather than the simple growth rate (dw/dt) (Fig. 13.2). As we have just seen, it is only in exceptional circumstances that this can be expected to be constant. Many formulae have been advanced, on a variety of grounds, in an attempt to produce a theoretical scheme which fits the facts better.

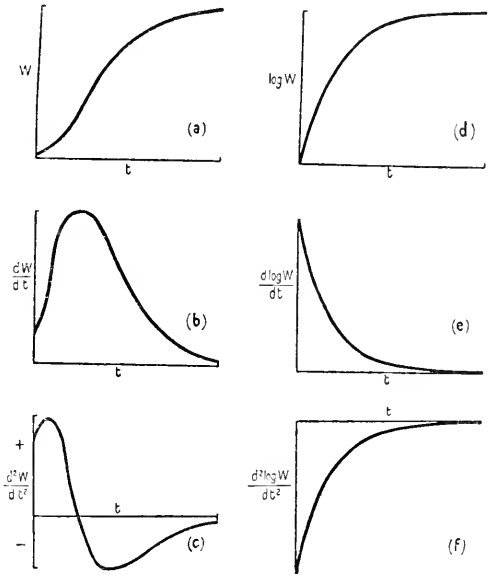


FIGURE 13.2

Various 'growth functions'. The three curves in the left side are concerned with the absolute growth. In *a* weight is plotted against time to give the curve of growth (a Gompertz equation is assumed); in *b* growth rate is plotted against time, and in *c* the change of rate (i.e. acceleration) of growth is shown against time. The curves on the right give similar graphs of the functions of the *specific* growth rate; that is, in *e* we plot 'growth rate per unit mass' or $\frac{1}{W}\frac{dW}{dt} = \frac{d \log W}{dt}$ instead of simple growth rate $\frac{dW}{dt}$, and make corresponding changes in the other curves *d* and *f*. Notice that the specific growth rate falls steadily from the beginning of life (curve *e*), and does so at an ever-increasing rate (curve *f*). (From Medawar 1945.)

It is worth while just to glance at the main varieties of these.

(1) The 'monomolecular' formula is derived from the idea that the growing substance is formed from a store of some substance a which is gradually used up. This gives the relation

$$\frac{dw}{dt} = k(a - w),$$

whence

$$w = a(1 - be^{-kt}),$$

when b is a constant depending on the initial amount of a.

When time is plotted horizontally, this gives a curve which is convex upwards and which approaches the upper limit a. Rather few growing systems behave in this way.

(2) The 'logistic' formula can be deduced from a number of different assumptions. For instance, if the substance w is formed from a precursor a as before, but if the rate of formation is also increased in proportion to the amount of w already present (i.e. if the reaction is 'autocatalytic'), we shall have

$$\frac{dw}{dt} = kw(a - w).$$

Or, if we suppose that the rate of formation per unit mass decreases in proportion as w is formed, we shall have

$$\frac{1}{w}\frac{dw}{dt} = p(q - w)$$

where p and q are constant, which amounts to the same thing.

The differential equation can be solved to give an expression of the form

$$w = \frac{a}{1 + be^{-ct}}$$

This when plotted gives a sigmoid curve, which eventually approaches the limit a.

(3) Another assumption is that $\frac{1}{w}\frac{dw}{dt}$ decreases as time goes on, but in a different way, being proportional to log w. This is the 'Gompertz' equation:

$$\frac{1}{w}\frac{dw}{dt} = p \log w - q.$$

This gives a solution of the form

$$w = ae^{-be^{-kt}}$$

which is again a sigmoid curve which approaches the upper limit a.

(4) Again, we can take it that $\frac{1}{w}\frac{dw}{dt}$ decreases simply in inverse proportion to the lapse of time

$$\frac{1}{w}\frac{dw}{dt} = \frac{k}{t},$$

$$\text{whence} \quad w = bt^k.$$

This is the 'parabolic' or 'double-log' curve; it should give a straight line when log weight is plotted against log time, whereas the 'exponential' or 'single log' formula $w = be^{kt}$, which holds when $\frac{1}{w}\frac{dw}{dt}$ is a constant, gives a straight line when log w is plotted against time. The double-log formula fits better with the weights of such growing systems as embryos, at least in the early stages, but like the single-log expression, it has no upper limit as time increases and can therefore obviously only hold for part of the life-history of most animals, which reach a final adult size. (It is possible that certain animals, including fish, continue growing indefinitely.)

All the formulae have been applied by various authors to the actual data derived by weighing embryos during their growth. Such observed growth curves are generally roughly sigmoid in shape, but they may give evidence of a number of cycles of growth, so that applying any one type of formula one may have to invoke a set of different constants for each cycle. Even so, it has never been possible to show that any one of the above formulae fits the facts so exactly that it must represent the actual situation and the others can be excluded. There are many snags in fitting theoretical curves to the actual observations. In the first place, many growth curves have been derived by weighing a sample population at each of a series of ages, taking the average of each age group, and joining the points together to give the overall curve. It is difficult to do anything else if we are interested, for instance, in the foetal growth of a mammal. But there will, of course, be a certain variation in the stage of development reached by individuals of the same temporal age, and if there were any sudden spurts or slowings-down of growth these might be obscured by taking averages. For instance, there is usually a sudden spurt in the growth of a boy at the time of puberty. In some boys this occurs rather earlier, in some rather later. If one derives a growth curve by weighing groups of boys at various ages, the spurt becomes distributed among a number of different groups and its existence concealed.

Even when a growth curve can be obtained by weighing a single individual at various times during its life, the curve is bound to suffer from a

certain lack of precision. There may have been unavoidable alterations in the environmental conditions, amounts of food, temperature, disease, etc., and each weighing will only be accurate within certain observational limits. Thus what we shall have as a basis for setting up the growth curve is not a set of absolutely precise points but rather a zone of greater or less width within which the curve must lie. It will always be possible to fit quite a number of different theoretical curves into such a zone, particularly if we allow ourselves to consider the possibility of a set of growth phases for each of which a new set of constants may be calculated. Thus it is extremely improbable, and in fact does not happen in practice, that a set of empirical observations can suffice to discriminate between the various theoretical possibilities.

Moreover, it is quite obvious that when dealing with the growth of an entity such as an embryo, we are not confronted with a 'growth' which corresponds to any precise definition. The embryo contains a highly heterogeneous collection of tissues, some of which are growing, probably at different rates, while other parts of the embryo, such as the blood plasma, are not growing in any normal sense at all.

Weiss (1949*a*) has given a very clear and vivid picture of the type of complexity which is involved, even in the growth of a single organ, such as the eye. He writes: 'The original eye vesicle consists of a certain initial allotment of cells from the embryonic brain wall. At first, all of these cells divide. The growth function at this stage is therefore a volume function. In the cup stage the retina becomes multilayered, with a sharp division into a germinal and a sterile zone. Only the cell layer in contact with the outer surface, corresponding to the ventricular (ependymal) layer of the brain, continues to proliferate, while the cells released into deeper layers differentiate the various retinal strata without further multiplication. The source of growth thus has become reduced to a two-dimensional one, causing a marked decline in the relative growth rate taken over the whole organ (e.g. from measurements of diameter). Later, the cells of the germinal layer themselves cease to proliferate and transform into sensory cells, a process which starts from the centre (macula) and spreads rapidly toward the periphery (ciliary zone) of the retina. Eventually, only the cells at the rim retain residual capacity to multiply. Further growth is then essentially by apposition from this rim; that is, the growth source has shrunk from planar to linear extension. Meanwhile some of the neuroblasts, though no longer multiplying, grow in size as they sprout nerve processes, which, grouped into plexiform layers, add to the thickness of the retina. During the later stages a gelatinous secretion, supposed to come from cells of both retina and lens, fills the interior with vitreous humour, thereby

progressively distending the eyeball. In addition, blood vessels and other mesenchyme penetrate into the eye from the surroundings.

'This diversity and complexity of the component processes contributing to eye size makes the search for a single "growth-controlling" principle appear utterly unrealistic . . .'

Further, it must be remembered that the growth of an organism or organ may be due to a multiplication of cells, which remain about the same size, or to an enlargement of cells which do not increase in number. The final number of muscle or nerve cells in a vertebrate, for instance, is probably attained fairly early in embryonic life, the growth of the organs thereafter occurring mainly, if not entirely, by increase in cell size. It is not entirely clear whether the two types of growth depend on quite different underlying synthetic processes, but there is obviously some considerable difference between them, so that there is no reason to expect that they would follow identical growth laws.

Finally, it is possible for organs, or even entire organisms of relatively simple structure, to 'de-grow' or become smaller. Flatworms or coelenterates may respond in this way to deprivation of food supplies. In higher animals, the regression of a tadpole's tail at the time of metamorphosis is a striking example.

It is hardly to be expected that any very simple formula can fit all such cases. Attempts have been made to elaborate more complex ones. Perhaps the most valiant is that of Wetzel (1937) who set up an equation of extreme complexity containing over a dozen different constants, each of which was designed to deal with one or other of the factors which he supposed to be involved in the growth of a heterogeneous collection of tissues, such as an embryo. The formula was so complex, and therefore so flexible, that it could have been made to fit almost any set of data. Its justification would in fact have to be sought not in the accuracy with which it could be fitted to observations of growth, but in the experimental justification of the various parts of the formula which were concerned with the postulated underlying processes. We still know far too little about the unit processes which go to build up the overall growth rate of an embryo for such an experimental justification to be provided.

Most authors recently have been content to accept the situation that the growth of a complex organism cannot either be formulated adequately in terms of any simple, global hypothesis, such as those listed above, nor can it as yet be adequately analysed into a series of part processes; whence it follows that we have to use the various growth formulae merely as convenient means of summarising the empirical observations without attempting to attribute any profound meaning to them. For further

progress in the study of growth as a precisely defined concept we shall have to look to investigations on the synthesis of definable and isolatable proteins, such as those of Spiegelman, Monod and others, on the rate of formation of adaptive enzymes (pp. 400, 409).

Meanwhile, empirical studies on the growth of the organism as a whole present many points of interest which, however, there will not be space to follow in any detail here.

One most interesting and technologically important aspect of the matter is in connection with the interaction between environmental and genetic factors in the determination of growth rate and absolute size. Very little indeed is known about the physiology of such process in animals, and they present a promising field for investigation. Some work of potentially fundamental importance is being made by the use of identical twin cattle. The members of such pairs of twins have exactly the same hereditary constitution. Bonnier, Hansson and Skjervold (1948) have shown that their growth rates, although considerably influenced by the genes, can be modified to quite an important extent by the level of feeding during the growing period, but that two identical twins, one reared with abundant nutrition and the other with much poorer supplies, will eventually tend to reach about the same final adult size although approaching this at different rates. Again, King (1954) finds that if a twin is kept for a period on a low-level diet and then changed to a high level, it soon makes up for any stunting it may have undergone, and proceeds to grow at the fast level characteristic of its abundant nutrition. After a certain period on the high diet, it will be heavier than its co-twin if the latter has been given the same diets in the reverse order, first high and then low.

The mechanisms controlling final size, i.e. the factors which cause an animal eventually to stop growing, are scarcely understood at all. Some species probably never cease growth; this is said to be the case for fish. Others stop at a certain size, although the tissues are still capable of growing, and will do so if a part of the body is amputated. Others again (e.g. mammals) grow till they reach a certain age, and, as we have just seen, tend to reach a characteristic limiting size in the growing period.

There may, perhaps, be no general mechanism which operates in all these different situations; if there is, it is still obscure. Moment (1953) has suggested that the limit might be set by the gradual building up of differences in electrical potential. Since tissues consist of cells, which are semi-permeable bags containing electrolytes among which active chemical changes are going on, it is to be expected that potential differences will exist; and they have in fact been detected (Review: Lund 1947). Moment

supposes that the extremities of an animal tend to become electro-positive and thus favourable to oxidations, and this, he argues, is inimical to growth. The evidence for the existence of such a situation (or for its effectiveness if it does exist) is not very strong. Perhaps a more plausible mechanism is to be found in auto-inhibitory effects, of an immunological nature, such as those postulated by Rose and others (p. 193).

Another aspect of the overall growth rate is its dependence on endocrine secretions, particularly those of the pituitary. There is not space here to deal with this subject, which belongs to endocrinology rather than embryology.

2. *The relative growth of parts*

It is obvious that the different parts of an embryo do not always grow at the same rate. Several different lines of attack on the problem have been followed.

Perhaps the simplest is that opened up by Huxley (summarised Huxley 1932, see also Medawar and Clark 1945, *Symp. Soc. exp. Biol.* 1948, Zuckerman 1950). He showed that if x is the magnitude of a whole organism and y that of some part of it, the relation between them can often be represented by the equation

$$y = bx^{\alpha}$$

or, what is the same thing,

$$\log y = \log b + \alpha \log x.$$

As the second equation shows, the two magnitudes will give a straight line when their logs are plotted against one another (Fig. 13.3*a*). There is no doubt that the formula does fit rather well to very many sets of data and is a very useful generalisation. The phenomena has passed under a variety of names, of which heterogony and allometry seem to be the most usual.

It is not at all easy to decide just what the formula means in biological terms. Taking it from the simplest point of view, we may say that b is a relatively unimportant constant, which specifies the size of the organ y when the whole organism x is unity. The other constant α is the one which relates to the rate of growth of the organ: if the growth rate of both x and y is proportional to their actual size we shall have

$$\frac{dx}{dt} = Ax, \qquad \frac{dy}{dt} = Bx,$$

whence $y = bx^{B/A}$, or $y = bx^{\alpha}$, when $\alpha = B/A$.

But if we adopt a more realistic formulation of the growth rates of x and y, making $\frac{dx}{dt} = f(x, t)$, when $f(x, t)$ is one of the functions discussed in the previous section, it can be shown that although the allometry formula is often a good approximation, it will only be exactly true in exceptional cases.

There are other reasons why the formula cannot be accepted as a strictly accurate description of the situation. The most important is that if two

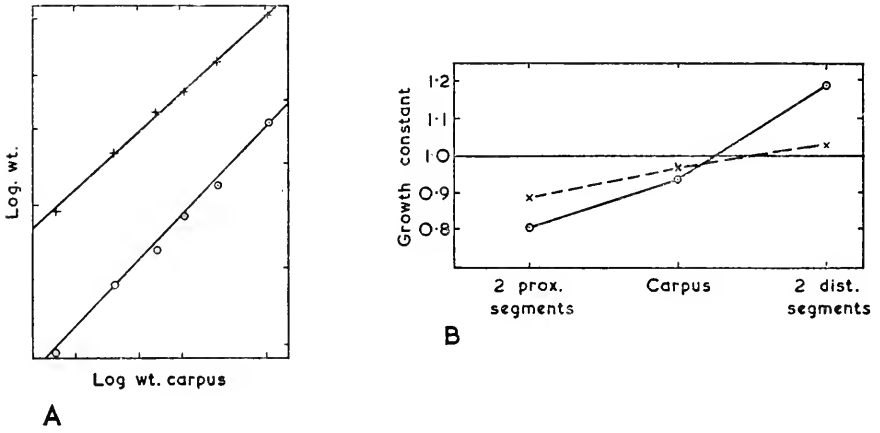


FIGURE 13.3

A, the log weight of the two distal segments (crosses) and the two proximal segments (circles) plotted against the log weight of the middle segment (the carpus) of the claw of the Fiddler crab *Uca pugnax*. The slope of these lines defines the allometric growth constants *B*, the gradient in growth constants along the claw in *Uca* (full line) and the spider crab *Maia* (dotted line). (After Huxley 1932.)

segments of an organ, y_1 and y_2, are each related heterogonically to the whole organism, then the sum of the two segments cannot be so related, since if $y_1 = b_1x^{\alpha_1}$ and $y_2 = b_2x^{\alpha_2}$ then $y_1 + y_2$ cannot be of the form bx^{α}, though the discrepancy is usually not very large.

One must conclude that the allometry formula, like the other growth equations discussed previously, can at best be taken as a useful empirical summary of a set of data, but that it is not a firmly based theoretical principle.

Even with this limitation, a number of conclusions can be drawn from it. In the first place, as long as α remains constant, the rates of growth of the two parts are preserving a constant relation to one another; and it is

a remarkable fact that they so often do so. One simple physiological explanation of such a system would be the hypothesis that the organs are each competing, with constant efficiencies per unit mass, for a generally available supply of nutrients. An attempt to test this has been made by Twitty and Wagtendonk (1940), who transplanted eyes of various ages and sizes between different individuals of axolotl, which were fed at different levels. They found that the simple scheme of constant efficiencies of competition was certainly not the whole story, since, for instance, a young eye transplanted to an older host which was starved might continue to grow even when the host was declining in weight. They concluded that the assimilative capacity of an organ must change (usually if not always decreasing) during the course of development (cf. p. 298). A similar conclusion emerges when one studies the growth of fragments of an organ isolated *in vitro*: pieces of chick heart, explanted from embryos of increasing age, show an increasing lag period before they start growing and a decreasing rate of growth in the first few days, though these differences fairly soon disappear (Medawar 1940).

Several authors, beginning with Teissier in 1931 and Needham in 1932, have applied the allometry formula to the increase in various chemical entities during development (reviewed in Needham 1942). If wet weight is plotted on double-log paper against dry weight, or glycogen, fat, protein, ash, calcium, phosphorus, etc., plotted against each other or against wet or dry weight, a series of straight lines are obtained. This indicates that the entities are related in the manner of the allometry equation

$$\log x = a \log y + \text{constant}.$$

The very interesting fact emerged that if we measure a number of different substances, x, y, z, etc., in two different animals A and B, we find relations of the kind

$$\log \frac{x}{A_1} = a \log \frac{y_b}{A_2} = \beta \log \frac{z_a}{A_3},$$

$$\log \frac{x_b}{B_1} = a \log \frac{y_b}{B_2} = \beta \log \frac{z_b}{B_3},$$

in which A_1, B_1, etc. are different constants, but a, β, remain the same, whatever the animals in which x and y are measured.

There is thus the same general relationship between a particular x and y (say fat and glycogen) throughout the animal kingdom or a great part of it. Needham spoke of this as a 'chemical ground-plan of animal growth'. Waddington (1933*c*) suggested that one might envisage the situation in terms of a general speeding up or slowing down of a basic chemical

programme. We can express the growth rates of entity x in animals A and B as two time-functions:

$$\log \frac{x_a}{A_1} = f_a(t), \qquad \log \frac{x_b}{B_1} = f_b(t).$$

Now we could choose another unit for measuring time, t^1, such that $f_a(t^1) = f_b(t)$. This would amount to measuring the growth rate of $\frac{x_a}{A_1}$ in time-units which made it identical with that of $\frac{x_b}{B_1}$. The important point is that this same transformation of the time-unit would automatically convert the growth rate of $\frac{y_a}{A_2}$ into that of $\frac{y_b}{B_2}$, and that of $\frac{z_a}{A_3}$ into that of $\frac{z_b}{B_3}$ etc. Thus one change in time-scale would convert the whole chemical growth system of one animal into that of another (apart from the complication due to the constants A_1, A_2, etc. which express the initial state of the system when growth starts). We have here an approach to a concept of 'biological time', by which is meant the notion that events in, say, a mouse or an elephant, are similar but are all uniformly speeded up in the former as compared with the latter. It is not yet clear to what sort of entities such a notion can be applied: for instance it seems most improbable that any such relation can hold for molecular enzymatic processes. Further discussions of it will be found in Brody (1937) and du Noüy (1936) and some highly critical remarks in Medawar (1945).

3. *Growth gradients and transformations of shape*

In a complex organ, it is often found that the growth rate, relative to some standard part, varies in a graded manner from place to place. The simplest expression of this can be seen when there is a series of more or less comparable parts, for each of which an allometric growth constant (a) can be ascertained. Huxley (1932, Reeve and Huxley 1945) has described many examples, relating for instance to the joints within a crustacean limb, or the series of limbs attached to the different segments of the body. Fig. 13.3*b* shows how the a's for the different segments fall into an orderly sequence, which can be taken as defining a growth gradient.

If one measures the growth constants for a series of distinct sub-units within an organ such as a limb, there are of course definite jumps in its value between adjacent segments, and what should, perhaps, be a continuous gradient, is described in terms of a discontinuous series of steps. Examination of other cases indicates that in fact the gradients within a single mass of tissue are usually, if not always, continuous in gradation.

One method of illustrating this was introduced by D'Arcy Thompson (1916), although he used it to compare adult forms which are evolutionarily related rather than ontogenetic stages of a single individual. He took one adult form as a standard, drew it in outline as seen when projected on to a plane, and superposed on the drawing a rectangular network. He showed that if this network is treated as a grid of co-ordinates, and is then distorted in the appropriate manner, the drawing of the original shape will be distorted at the same time into a fairly good outline of some other type of animal (Fig. 13.4). Such distortions of a co-ordinate network will produce alterations which are continuously gradated over the whole area, and thus the growth gradients which are affected are probably also continuously graded, since they are likely to depend on the same fundamental mechanisms as produce the distortions which differentiate one form from the other. More recent workers have in fact shown that the same method can be used to compare different developmental stages of a single species.

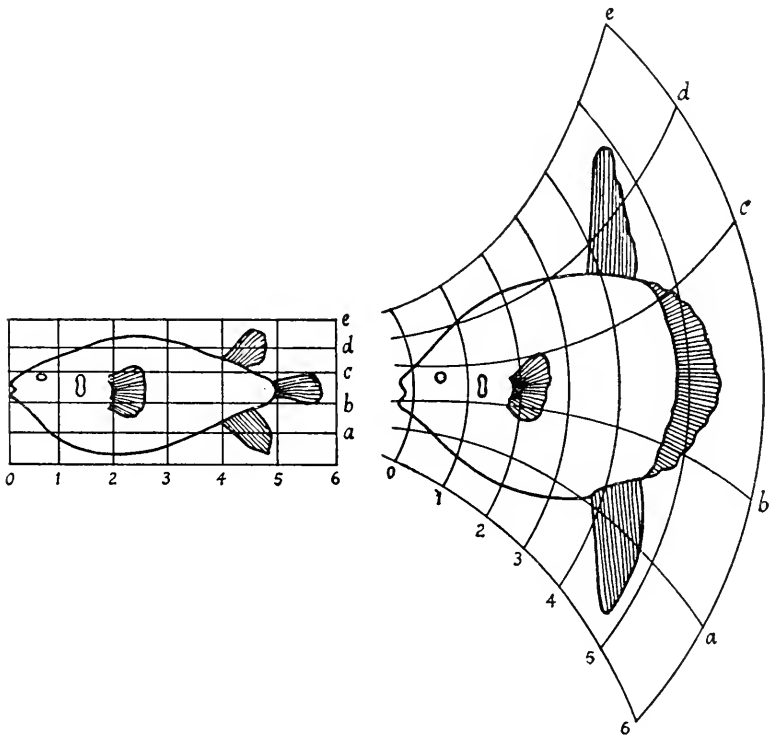


FIGURE 13.4

A transformation of a co-ordinate grid which converts the outline of *Diodon* (left) into that of its relative the sunfish *Orthagoriscus*. (After D'Arcy Thompson 1942.)

D'Arcy Thompson's suggestions opened up a large field for investigation, but unfortunately this has not been as systematically studied as might have been hoped. We can deal with the recent work under three heads: firstly, improvements in the method; secondly, the general physiology of growth gradients and shape transformations, and thirdly, attempts to discover the physiological mechanisms underlying them.

Actually rather little has been done to make D'Arcy Thomson's method of the distortion of a co-ordinate network into a means of exact analysis. Medawar (1944, 1945) has made some steps in this direction (see also Richards and Kavanaugh 1945). He considered the changing shape of the human body during its development from the early foetus to the adult. The body was first reduced to a two-dimensional shape by representing it as a series of outline drawings when seen from the front (Fig. 13.5). It becomes obvious then that in the early stages the legs grow faster than the parts nearer the head, and it appears probable that there is a single continuous growth gradient with its high point towards the feet, falling off as one goes higher up the body. Medawar pointed out that this could be represented by a transformation of co-ordinates and that this transformation could theoretically be specified in algebraic terms. To illustrate how this might be done he reduced the shape of the whole living body still more drastically and considered only certain points on the vertical midline; the foot, fork, navel, nipples, chin, etc. The original three-dimensional shape was thus reduced to a line on which certain intervals are marked. Suppose now that P_1, P_2, P_3, etc. are the heights from foot to fork at successive points in time, and similarly Q_1, Q_2, Q_3, etc. the heights from fork to navel at the same times, and so on for the other intervals. We can from the actual measurements work out empirical equations connecting the successive *P*s and again, another set of equations connecting the successive *Q*s, and so on. Each equation will give the changes of one part of the network as time proceeds. We can also find algebraical relations between the equations relating to the *P*s and those relating to the *Q*s, the *R*s and *S*s, etc. at any given point in time. With the aid of these two sets of equations, the whole series of transformations can be expressed algebraically. It is clear, however, that quite a lot of arithmetic is required to produce even a fairly clumsy algebraical description of a series of shapes, notwithstanding that these have been reduced to their very simplest form, the original three dimensions having been whittled away to one. Such labour is only justified if it enables one to see certain relations which would otherwise be missed. So far, such evidence of a real usefulness of the method has not been forthcoming.

There are contexts, however, in which it seems probable that Medawar's

methods would be useful if they were applied. The changing shape of animals as they mature is of practical importance in relation to the production of livestock. Hammond (1950) has been particularly concerned with the problem and has demonstrated a number of general points about the physiology of growth gradients. These he has been able to illustrate pictorially, but it has so far not been easy to reduce them to a precise and manageable form; Medawar's methods might be very useful in this connection.

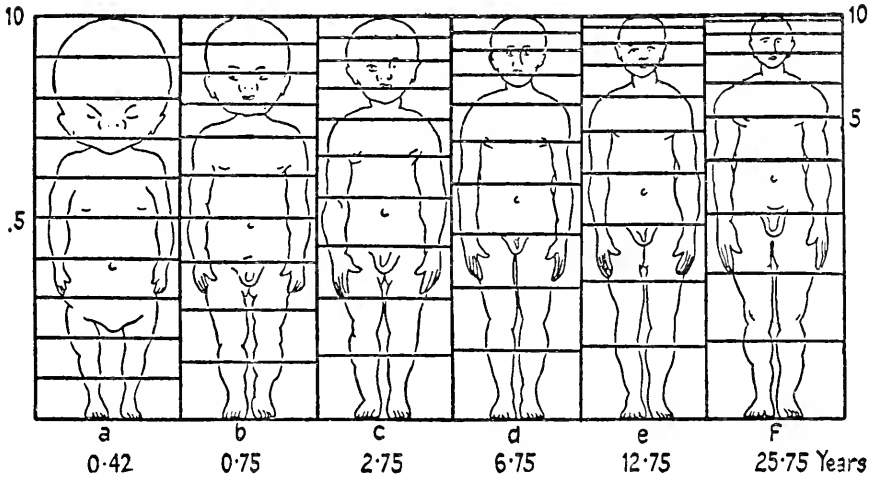


FIGURE 13.5

Changes in the proportions of the human body during growth. The heights of certain landmarks (knees, fork, navel, mouth, etc.) were ascertained for each age, and related to one another by means of empirical equations. From these equations the heights were recalculated, to give the horizontal lines which are drawn on the figures. The goodness of fit of these lines indicates the degree to which the changes in proportions have occurred in a regularly graded manner, so as to lend themselves to summarising in relatively simple algebraic functions. (From Medawar 1945.)

Hammond shows that the various species of wild animals from which our domestic livestock have been derived have each their characteristic pattern of changes in shape during development. These can be illustrated rather vividly if one takes as a standard an early developing part such as the head and shows a series of drawings or photographs of different stages of the animal, all of which have been adjusted to the same head size. We then see that in the horse, for instance (Fig. 13.6), up to the time

of birth there is a great decrease in the length of leg relative to the remainder of the body, whereas after birth the most important changes are a lengthening and deepening of the body. In wild sheep the changes are somewhat similar, though perhaps not so marked. In wild pigs there are no very marked changes in proportions from the foetus up to the adult. It is these basic developmental patterns which are altered either by the genes selected by the livestock breeder or by the conditions of feeding

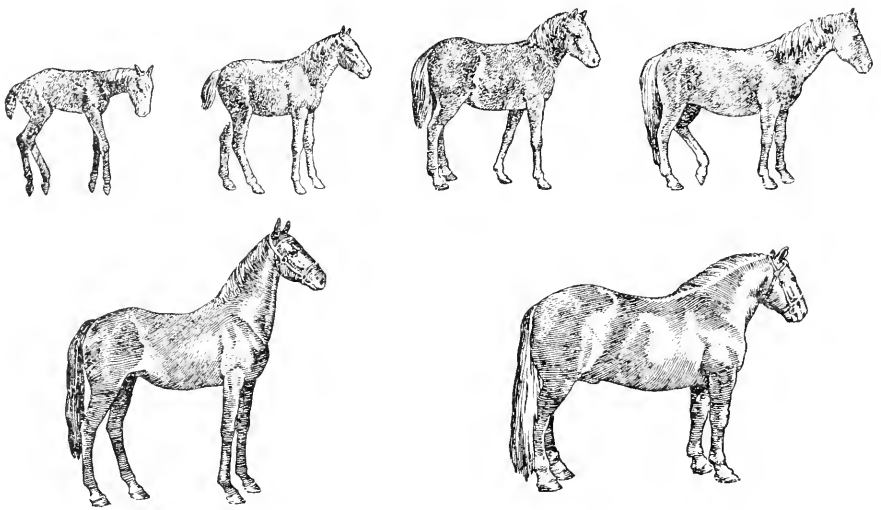


FIGURE 13.6

Changes of proportion in the growing horse. The upper row of drawings show a primitive type of horse at various stages from the foetus to the adult, adjusted in size so as to have the same length of cranium. By the stage of the first drawing (late foetus) there has already been a great development of the legs; later there is a preponderant growth of the trunk. The two drawings below show on the left a thoroughbred, and on the right a draught-horse; the later phase of growth has been minimised in the former and enhanced in the latter. (After Hammond 1950.)

and husbandry under which he keeps his animals. Among horses, for instances, the strains bred for speed have been produced by selecting genes which partially suppress the later changes, so that the adult horse retains the long legs and slim body of the normal juvenile phases. In the heavy draught horses, on the other hand, genes have been selected which increase these later changes so that one obtains an animal with a very large and heavy body and relatively shorter legs. In the mutton breeds of

sheep it is again genes which encourage the later changes in body conformation which are required and in the improvement of pigs it is also the late-developing hindquarters rather than the early-developing head and forequarters that it is necessary to emphasise.

The changes in body proportions can be affected not only by genes but also by the level of nutrition on which the animals are kept. For instance, if two comparable sets of pigs are kept, one on a high plane of nutrition and another on a low plane, until they both reach the same weight, it will not only be found that the high-plane pigs reach the specified weight more quickly, but that the two sets of animals differ in conformation at the end of the experiment. The low-plane animals retain a more juvenile shape. One can say that in spite of maturing more slowly they mature in a conformation which is characteristic of a younger animal than do the similar pigs reared on the high plane of nutrition. Hammond explains the situation in the following ways: The different regions of the body, such as the head and the forequarters, the hindquarters, etc., attain their greatest rate of relative growth in succession. The same is true of different tissues, such as bone, muscle, fat, etc. The fundamental sequence in which these various parts come to the fore is never altered, but changes in genes or changes in nutrition can either compress the sequence into a shorter length of time or spread it out over a longer interval. High nutrition brings the successive phases nearer together in time; so do the genes which determine the draught type of horses or the meat-producing type of sheep or cow. Low nutrition spreads the phases further apart and so do the genes for racehorses (Fig. 13.7).

It appears from other experiments of Hammond and his associates that even if the plane of nutrition restricts the outward expression of the sequence of phases the basic physiological changes determining them may be proceeding nevertheless. Thus two sets of pigs were brought to the same weight in the same length of time, but by different routes; one being kept first on the high plane and later on a low, the other first on a low and later on a high plane of nutrition. They nevertheless showed characteristic differences in conformation. There is obviously a great deal more work to be done on the physiology and the genetics of these relations. It is one of the most fascinating, and also most practically important, aspects of the whole subject of growth.

Our knowledge of the mechanisms which control growth patterns is very meagre. We have to recognise in the first place that the processes which finally issue in an adult shape may be very complex. Waddington (1950*a*) has discussed one particular case from this point of view, that of the wings of *Drosophila* and shown how the final shape depends not only

on successive phases of cell division and cell expansion but also on deformations of the whole structure resulting from the changes in pressure of the body fluid contained in it. No very simple physiological account of the general growth pattern can be expected in such complex cases.

Even when we are dealing with a simpler case, in which the dominant factor is growth in the ordinary sense of cell multiplication and cell enlargement, our present knowledge does not provide a basis from which the phenomena can be easily understood. The growth pattern seems to

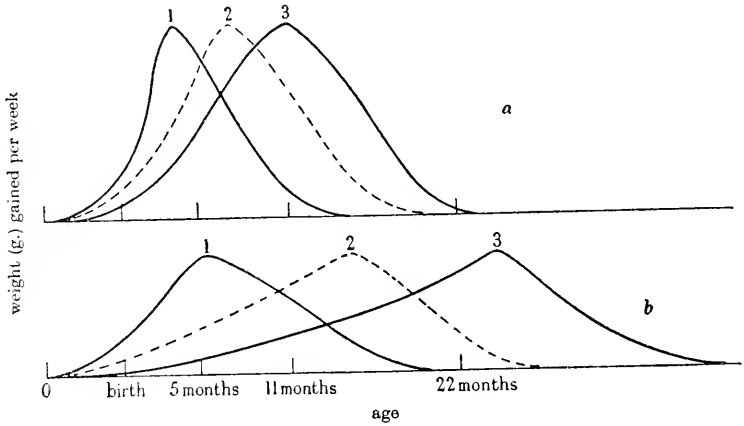


FIGURE 13.7

Diagrammatic curves illustrating the succession of anatomical systems which are predominant in growth rate in mammals (particularly farm livestock). Curve 1 relates to the cranium and shanks, bone and gut-fat, which have a high growth rate at an early stage; the neck, main body musculature and subcutaneous fat grow fastest at a rather later stage (curve 2), and the hind-quarters and intra-muscular fat still later (curve 3). The upper set of curves show the situation when the animal is kept on a high plane of nutrition; the phases follow one another rapidly. Under conditions of poor nutrition (lower curves) the succession is more long drawn out. (After Hammond 1950.)

characterise whole regions, which may be made up of a number of anatomically different elements, and it is surprising how often these elements appear to work together in a harmonious way. Fig. 13.8 shows in profile the skulls of a number of different types of dogs. The differences are presumably genetically determined and it is clear therefore that genes affect the pattern as a whole and not only the individual units comprising it. Moreover it must be remembered that each skull is made up of a number of different bones; to mention only the most striking example, the lower

jaws are usually modified in a way consistent with the shape of the upper part of the skull to which they are attached. This subordination of individual parts to the whole to which they belong is the general rule, but it is not quite universal. For instance, in the F_2 and later generations of certain crosses between different breeds of dogs, some cases of definite disharmony between upper and lower jaws and between other parts of the body may be found, but they are relatively rare. Co-ordination usually extends not only between parts of the same general nature, such as bones, but affects tissues of quite a different kind, such as skin, muscle, etc. It is, however, rather commoner to find instances in which the growth rates of markedly different tissues are not properly assimilated to one another. For instance, in dogs with greatly shortened faces, such as bulldogs, the skin is often too large for the bony structure and therefore has to hang in folds (Stockard 1941).

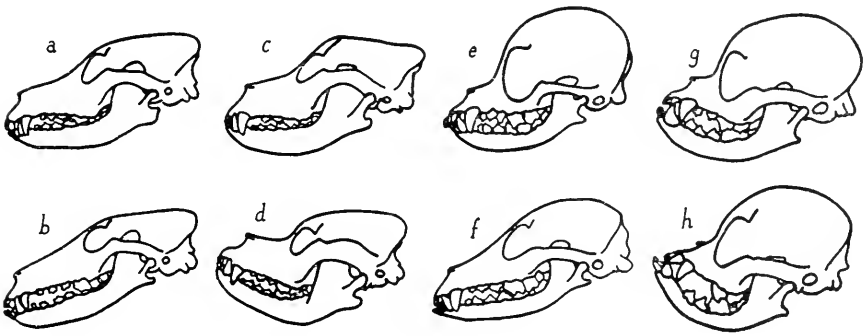


FIGURE 13.8

Skulls of various races of dogs, adjusted to the same size, to illustrate how the structure, although complex, is modified as a whole. (*a*) German sheep dog; (*b*) Borsoi; (*c*) German Dogge; (*d*) St. Bernard; (*e*) Zwergspitz; (*f*) Italian Windspiel; (*g*) Englischer Mops; (*h*) Japanese spaniel. (After Huber 1948.)

Very little is known about the ways in which such growth correlations arise. General endocrine control affecting all types of tissue certainly plays a part in many cases. It seems probable also that the growth rate of one organ or part of an animal usually has some influence on the growth rate of the neighbouring regions. For instance, Huxley (1932) compared the growth rate of the limbs in Crustacea in species in which one sex has a very large, fast-growing limb, which is absent in the other. He showed that the presence of a fast-growing limb tended to affect its neighbours, in

general increasing the growth of the limbs immediately posterior to it and depressing the rate of the limbs immediately in front. The mechanism of the effect—whether the fast-growing limb operates by secreting some growth-promoting substance or by competing in some way for a limited supply of available raw materials—is quite unknown.

One of the most extended series of studies of the physiology of growth rate of individual organs is that by Harrison, Twitty and others on the eyes and other organs of the Mexican salamander *Amblystoma* (Reviews: Harrison 1933, Twitty 1934, Needham 1942, Reeve and Huxley 1945). The eye of one species, *A. tigrinum* (the normal axolotl) is very much faster growing than that of the nearly related species *A. punctatum*. It was first shown that if the eye-cups are interchanged and transplanted from *punctatum* to *tigrinum* or vice versa, each type retains its own characteristic growth rate even in the new situation. The growth rates are therefore inherent in the eye-cups themselves.

When organs are grafted into other animals of the same species but different age, the growth rate of a transplant younger than the host is speeded up, and that of an older one slowed down, until the grafted structures have reached a size appropriate to the body in which they lie. One hypothesis to account for this would be that the young organs have a greater assimilative efficiency than older ones, and are therefore able to obtain more than their normal share of nutrients from the blood stream when they have only older organs to compete with. But that can hardly be the whole story. Even when the competitive demand for nutrients was increased as much as possible (by starvation, to such a point that the host body lost weight, together with amputation of the tail which caused this to regenerate) the young transplanted eyes were still able to grow. Twitty came to the conclusion that at least two factors must be involved; not only a decreasing assimilative efficiency of an organ as it ages, but also an increase with age of the richness of the nutritive supplies in the blood (Twitty and Wagtendonk 1940). These ideas and observations are obviously closely related to those of Hammond mentioned above.

In other experiments by Twitty, the two main elements of the eye—the eye-cup and the lens—were combined in different ways. Here there was definite evidence of the influence of the growth rate of one element on that of the other. For instance, if a large eye-cup of *Amblystoma tigrinum* is combined with the small lens of *A. punctatum* or is allowed to induce a lens in *punctatum* skin, we find that in the compound eye, the eye-cup of *tigrinum* grows more slowly than usual while the lens of *punctatum* grows more rapidly, so that the two finally come to bear a

harmonious relation to one another. The same kind of thing is true in the opposite combination of a small *punctatum* eye-cup with a large *tigrinum* lens; the growth-rate of the eye-cup is increased and that of the lens decreased until the two fit. Again the mechanism of the effect is quite obscure. Weiss (1949*a*) has suggested that it may be a matter of tension exerted on the growing edge of the retina. In a combination where the lens is too small, it will permit some of the vitreous humour to flow out of the eye-cup and thus the tension will be reduced and the growth rate lowered. On the other hand, an over-large lens will exert a radial pressure against the edges of the retina and this might lead to an increased growth rate (Figs. 13.9, 13.10).

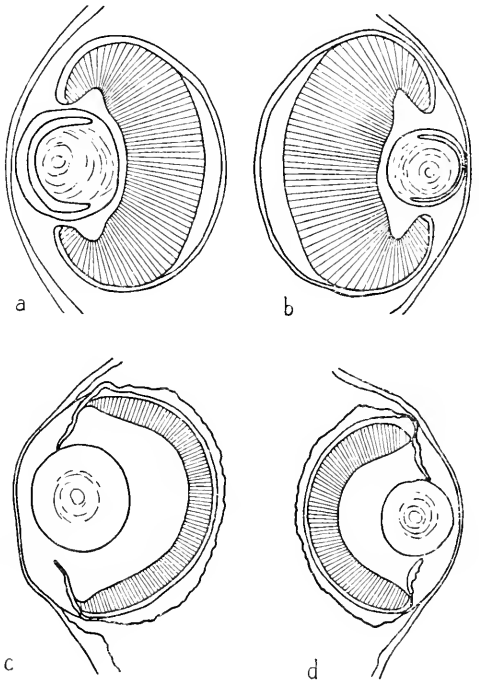


FIGURE 13.9

Reciprocal effects of lens and eye-cup. Figures *a* and *b* show the eyes of a fairly young tadpole of *Triturus taeniatus* in which the lens of the left eye (*a*) has been induced out of axolotl ectoderm grafted into the region; the lens is much too large in relation to the eye-cup. Figures c and *d* show a later stage from a similar experiment (at a lower magnification); the lens derived from axolotl tissue (in *c*) is still larger than the corresponding *taeniatus* lens, but it has caused its associated eye-cup to grow faster than normal, so that the relative sizes are nearly adjusted to one another. (After Rotmann 1939)

Many other combinations between tissues from different species of Amphibia have been made experimentally. They often exert influences on one another's growth but these differences are not always mutual. Rotmann (1933) for instance, found that if on the body of a newt of the species *Triton taeniatus* a limb was provided with a core of mesoderm of the

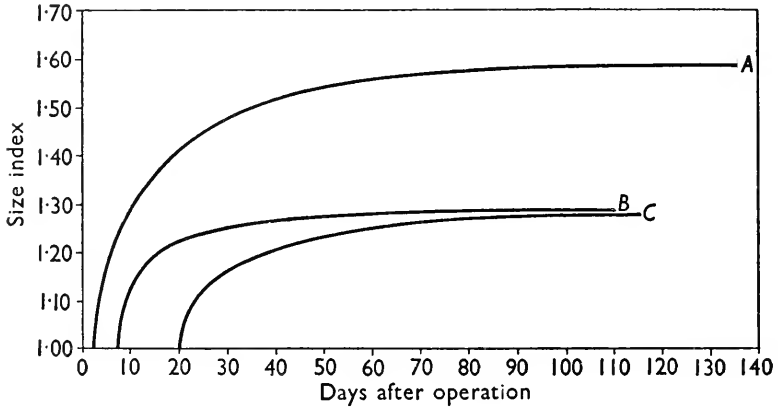


FIGURE 13.10

Relative growth of eye-cup and lens in inter-specific grafts in *Amblystoma*. Curve *A*, growth of *tigrinum* lens when associated with *tigrinum* eye-cup; curve *B*, growth of *tigrinum* lens when associated with *punctatum* eye-cup. The size of the lens is expressed as the ratio *tigrinum* lens to *punctatum* lens of same age. Note that the *tigrinum* lens in its own eye-cup reaches about 1·60 times the *punctatum* size, but when combined with a *punctatum* eye-cup only about 1·30 times. Curve *C* shows the growth of a *punctatum* eye-cup provided with a *tigrinum* lens, the 'size index' being its ratio to a normal *punctatum* eye-cup. Note that it becomes larger than usual, to about the extent required to fit the associated *tigrinum* lens. (After data of Harrison.)

species *T. cristatus*, but enclosed in skin belonging to the host's species, the form of the limb up to the time of metamorphosis was entirely dictated by the species that contributed the mesoderm, which imposed its own growth rate on the ectoderm without being in any way influenced by it (Fig. 13.11). Interesting also in this respect are the experiments of Baltzer (see 1952*a*) who, however, was concerned not only with the growth rate in organs compounded out of combinations of tissues from two different species, but even more with the original induction of the organs and the laying down of their basic structure. On the whole he

found that mesoderm was more likely than ectoderm to react to influences from its surroundings and become assimilated into a compound organ.

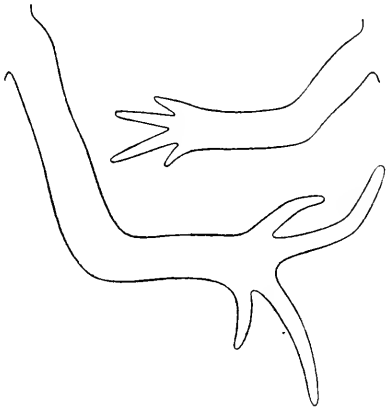


FIGURE 13.11

The two forelegs of a newt, *T. taeniatus*, in one of which (the lower in the figure) the mesoderm of the limb-bud had been replaced with tissue from *T. cristatus*. The operated leg has developed the long, rapidly growing toes typical of *cristatus*. (From Rotmann 1933.)

SUGGESTED READING

Medawar 1945, Reeve and Huxley 1945, Weiss 1949*a*, Zuckerman 1950.

CHAPTER XIV

REGENERATION

THE WORD 'regeneration' is used to refer to the processes by which an animal restores, or tends to restore, any regions which may be removed. It covers a wide range of phenomena (General Review: A. E. Needham 1952). At one extreme an adult mammal which has suffered the loss of a small part, such as a finger, or a larger part, such as a limb, can do no more in the way of regeneration than merely repair the wound and close the cut surface. At the other extreme a very small part of the normal body of a coelenterate, a flat worm, or a starfish, can restore the whole large region which is missing and become a complete individual. There are all grades in between these two extremes. The power of regeneration is in general greater in lower forms and less in more highly evolved ones, but this rule is only very rough, and when one looks at the matter in more detail it becomes apparent that the capacity for regeneration is distributed in a rather arbitrary manner throughout the animal kingdom. Often even closely related forms differ considerably in their regenerative powers. The mass of information on the subject is very large and there would not be space here to review it completely. It is, however, necessary to take a glance at certain aspects of the subject, particularly because of the light it throws on two general points of embryological theory. These are, firstly, the reversibility of determination, and secondly, the field theory.

Workers on regeneration (particularly Morgan in 1901), have distinguished two different modes in which the phenomenon can occur. If a part is removed from some organism, what is left may remain unaltered and regeneration be effected by the outgrowth of a new mass of tissue which becomes modelled into the missing parts. Such a process was called epimorphosis by Morgan. The word 'regeneration' is sometimes used in a narrow sense to refer to it alone (e.g. by Child, who employs 'reconstitution' as the more general term). In the other mode of regeneration, the part of the organism which is left after the amputation itself becomes remodelled so as to be transformed from a part into a whole organism. To this Morgan gave the name 'morphallaxis' which is still in common use.

Epimorphosis can certainly occur with little or no sign of morphallaxis. It does so, for instance, when a young newt regenerates an amputated limb, or an earthworm an amputated head, but it is not quite so certain

that morphallaxis ever occurs without some preliminary epimorphosis. For instance, if a flat worm is cut transversely in half some distance behind the pharynx the posterior half will eventually become transferred into a complete worm and this involves the appearance of a new pharynx some distance posterior to the cut. But it is probable that the first step is the appearance of a small outgrowth from the cut edge and that it is only after this has formed a new anterior part of the worm by epimorphosis that morphallaxis begins to occur and to cause the remodelling of the rest of the posterior portion.

Regeneration is often incomplete, less being formed than is necessary to replace what has been lost. For instance, a regenerated leg in the salamander may lack one or two of the toes, or the regenerated tail in a lizard show defects in its bony structure. There is perhaps nothing very surprising in this. It is more astonishing that animals should be able to regenerate at all than that they should sometimes fail to do so perfectly. It is more unexpected to find that there are also cases of super-regeneration. It is by no means uncommon for a missing part to be restored in duplicate. In some ways very closely connected with this is a phenomenon which can be regarded as regeneration without any previous loss to account for it. This is the process known as 'budding' in which a group of cells at some point in a complete normal animal suddenly start proliferating and develop into a new individual. The process occurs in groups such as the coelenterates and ascidians, when it gives rise to colonies of individuals which often remain closely attached to one another. In some worms, too, a head begins to form in the posterior region of the body and eventually the whole hind end breaks away and becomes a new individual.

1. *The origin of regeneration cells and their potentialities*

When a part of an animal is removed there are three sources from which the cells which build up the regenerate may be derived: (1) the tissues of the stump may grow out and form the new organ, retaining their original histological character and altering only in so far as they become part of the new region of the body (we shall use the word 'stump' in a general sense, to mean the region in the immediate neighbourhood of the place from which the organ has been removed); (2) the body may contain a reserve of undifferentiated or embryonic cells which accumulate at the wound and then differentiate into the tissues of the regenerate; (3) some of the already differentiated tissues of the stump may lose their differentiation and return to a more plastic condition from which they are able to redifferentiate into the specialised tissues of the regenerate, suffering during the process a greater or lesser change in their histological

character. The mass of cells which gather at the position of the wound, and which eventually develop into the regenerated part, is known as the 'regeneration blastema'.

It is probable that in morphallaxis the first process plays an important role. In general, however, it is by no means the most important of the three. Even in animals such as coelenterates, flatworms and oligochaetes in which morphallaxis occurs to a considerable extent, it has been shown that the body contains a supply of undifferentiated cells (known as reserve cells or neoblasts) which play a large part in the regeneration (Fig. 14.1). The stimulus of the wound activates these cells. In the coelenterates those

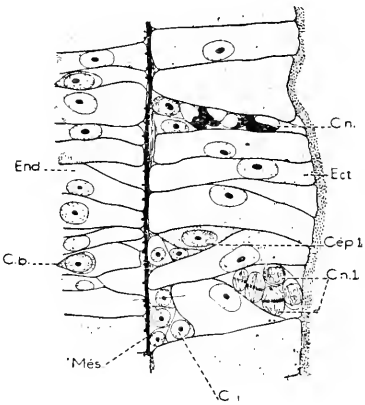


FIGURE 14.1

Longitudinal section through the gastric region of *Hydra*, with endoderm on left and ectoderm on right and the mesoglea (*Mes*) between. A group of neoblasts or 'interstitial cells' is shown at *C.i.* At *C.ep.1* similar cells are developing into epidermic (=ectodermal) cells, at *C.n.1* into cnidoblasts, and at *C.b.* into endoderm. (From Stephan-Dubois 1951.)

in the neighbourhood of the wound begin to divide and to produce the tissue out of which the regenerate is formed, while in flatworms the neoblasts even from further away migrate to the wound surface and give rise to the blastema (Stéphan-Dubois 1951). In higher animals such as vertebrates, the direct outgrowth of the already existing tissues is only a minor factor in regeneration. However, the nerve supply of a regenerate is probably always produced in this way, by the sprouting and outgrowth of the nerve fibres in the stump.

There has been a great deal of discussion about the origin and potentialities of regeneration cells in vertebrates, and there is still by no means

full agreement on all points. It may be pointed out in the first place that the regeneration process falls into three fairly distinct phases; (i) initiation of the process, and accumulation of a blastema; (ii) growth of the blastema, and (iii) differentiation. These phases can be differentially affected by various means. For instance, Lehmann and Bretscher (1952) found that certain amino-ketones inhibit the first phase (as does x-raying), while colchicine affects mainly the second, and quinoxaline derivatives the third.

The most important aspect of the initiation process is the production of the apparently undifferentiated cells which make up the blastema. This is usually a response to the injury involved in the amputation, but it can sometimes be brought about by means other than wounding. For instance, a number of Russian authors, beginning with Nassonov, have claimed that the formation of supernumerary limbs (by processes which can be considered as equivalent to regeneration) can be stimulated by injecting various tissue extracts and autolysates into otherwise uninjured axolotls, cartilage extracts being particularly effective (see Fedorov 1946). It seems likely that similar substances, released from the injured cells at the surface of the wound, are important in initiating blastema formation in the normal case. In adult Anura, under ordinary circumstances regeneration is either very slight or does not occur at all; and this seems to be due to an incapacity of the animal to produce a blastema. If a wound surface in such an animal is treated with strong sodium chloride solution (Rose 1944) or is lacerated mechanically (Polezhayev 1946), blastema cells appear and a considerable amount of regeneration may occur. Denervation of a limb in the urodele amphibian, on the other hand, usually leads to a failure of blastema formation and thus of regeneration (cf. Rose 1948*a*).

The next question to consider is whether the blastema cells in vertebrates are derived from reserve undifferentiated cells or from the dedifferentiation of the tissues in the near neighbourhood of the wound. Experiment has now demonstrated that the second of these is by far the most important source of the regenerating cells, although a minor contribution may come from the relatively undifferentiated connective tissues near the cut. The local origin of the cells can be demonstrated as follows: A limb is removed from a normal diploid urodele and a similar limb from a haploid specimen of the same species is grafted into its place. After union is complete the limb is amputated again, leaving only a small segment of the haploid limb attached to the stump. The regenerated limb is then found to be haploid and must have been derived from the cells of the small haploid segment (Hertwig 1927). Again it is a very general observation that irradiation with x-rays decreases an animal's capacity to regenerate

(cf. Brunst 1950), chiefly because it produces chromosome rearrangements which, when mitosis occurs, lead to unbalanced and inviable nuclear constitutions. Thus x-radiation can knock out the reserve cells of a planarian while leaving the animal as a whole capable of living but no longer of regenerating. If only the anterior region of a worm is irradiated and then a part of this region removed, regeneration is delayed for the time that it is necessary for the reserve cells to move from the non-irradiated region through to the cut surface (Wolff and Dubois 1948). Experiments of this kind in newts have given no evidence of any extensive movement of cells to form the regeneration bud in vertebrates.

It appears therefore that a regenerated organ in a vertebrate is in the main constructed out of dedifferentiated cells from the tissues close to the cut surface. New cells accumulate on the surface in a densely packed group to form a regeneration bud or blastema. Histological examination of the process gives evidence that muscles cells, bone-forming cells and other mesodermal elements contribute to the undifferentiated mass (cf. Manner 1953). Recent studies by Rose (1948*b*) also suggest that dedifferentiated epidermal cells make a considerable contribution. In the blastema all the cells lose their characteristic histological appearance. The important question arises whether this is a true dedifferentiation, and the cells able to develop again into something other than they were originally, or whether it is a deceptive appearance similar to that which can be seen in tissue culture, where cells from differentiated tissues lose their characteristic appearance and make an impression of being dedifferentiated, but in reality retain their specific nature and can develop again only into what they were before. The evidence suggests that to some extent at least the formation of a regeneration blastema involves a true dedifferentiation.

Perhaps the most conclusive evidence for the occurrence of true metaplasia (a change of character from one differentiated type to another) comes from the rather special case of the so-called Wolffian regeneration of the lens (Reyer 1954, J. Needham 1942). If the lens is removed from the eye of an amphibian the edge of the retina begins to grow and forms a group of cells which differentiate into a new lens replacing the old one. It seems quite clear here that differentiated retinal cells change their character completely to give rise to the final lens. This very odd type of regeneration has been known since the 'nineties of the last century when it was discovered by G. Wolff, after whom it is named. The retina does not need to suffer any wounding to become stimulated to start regenerating; this occurs as soon as the lens is removed even if the retina is quite uninjured in the process. It has been clearly demonstrated that the stimulus is chemical. The lens apparently gives off some substance which

holds the retina in check, and regeneration begins as soon as this is removed. Within the retina there is a gradient in readiness to undergo regeneration, the dorsal region showing greatest capacity and the ventral the least.

The situation is not so clear in the more usual types of regeneration, such as that of the limb or tail. It has been claimed by many authors (e.g. by Weiss 1930), that the blastema is at first quite undetermined and is competent to develop into almost any part. The first part of this claim seems to be justified. For instance, when early blastemas from regenerating limbs are transplanted into an indifferent situation, such as the body cavity of an adult salamander, they form masses of undifferentiated cells, which appear similar to malignant tumours (Waddington 1940*a*); it is probable, however, that they do not become fully malignant but are eventually encapsulated and keratinised. If a similar blastema is transplanted from a limb to the cut surface of an amputated tail, Weiss originally claimed that it would become determined by its new surroundings and thus differentiate into a tail; but this interpretation has been challenged on the grounds that it is difficult to be certain that the transplanted blastema was not simply resorbed, the tails which eventually appeared being formed not from grafted tissues but by the stump of the tail in the normal way. It would seem that the question could be settled by using polyploid or other specifically recognisable tissues to provide the transplant, but this has not yet been successful; May (1952), who tried it, found it impossible to recognise the cells of a triploid transplant in the redifferentiating tissues of the regenerate. Most authors who have reviewed the subject recently (e.g. J. Needham 1942, A. E. Needham 1952) reach the conclusion that the weight of the evidence is against the possibility of changing the fate of the blastema from one organ to another.

In contrast with this, there is a good deal of evidence that cells of the blastema can differentiate into any of a number of different types of *tissue*. Thus the power of regeneration can be removed from an amphibian tail or limb by x-raying; then one particular type of tissue from a normal limb can be grafted into it, and a few days later the limb amputated in the region of the graft. Luther (1948) claims that under these conditions, leg skin transplanted to an x-rayed tail gives rise to blastema cells which form all the tissues of the appendage (muscle, bone, blood vessels, etc.) but that they showed a tendency to form legs instead of tails. Trampusch (1951) has described similar evidence of changes of tissue specificity following transplantation of healthy skin, muscle or skeletal tissues to irradiated limbs. A converse type of evidence was obtained at a much earlier date by Weiss (cf. 1930), who showed that if one of the long bones

is removed from a limb, which is then amputated through the defective region, the regenerate will be provided with bone, although the bone will not be replaced in the region of the old limb from which it was excised (but this situation does not seem to hold for the tail, since if the axial organs are removed from that organ, amputation is followed by the regeneration of a structure with the same defect as the stump [Vogt 1931]). Finally, Schotté (1940) has claimed that if a very young regeneration blastema is transplanted into a situation in which it is exposed to the appropriate inducers, it may be caused to differentiate into lens or ear tissue.

We seem therefore to be driven to the rather unexpected conclusion that, although the cells of the early blastema are very labile in their histological properties, and can become almost any tissue (except probably nerve), there is little evidence that they can change their organ-specificity. If this is so, then, for instance, tail epidermis can undergo a 'metaplasia' by which it becomes converted into muscle—but it will be tail muscle even if the process occurs after transplantation to a site on the limb. Since organ-specificity is a rather unfamiliar concept, and we have no clue as to its chemical basis, it is surprising to find it obtruding itself in such a definite manner in experiment. There are, however, other contexts in which it appears that organ-specificity is a rather distinct character in the later stages of development. For instance, in birds the mesodermal core of a feather papilla can induce epidermis to develop into a feather germ; and the type of feather eventually produced depends strictly on the region of the body from which the competent epidermis comes, breast epidermis always forming breast feathers, saddlee pidermis saddle feathers and so on (Wang 1943).

The evidences of histological metaplasia are of fundamental importance for our understanding of the process of determination. They show that determination which occurs in embryonic stages, and the high degree of histological differentiation which follows it, need not involve absolutely irreversible changes, although shortly after the period of embryonic determination no means are known to bring the cells back to a plastic condition. Later in life the stimulus of wounding may have this effect. It follows that those nuclear genes which are concerned in the differentiation of the new histological type to which the cells switch over, must have persisted throughout the earlier period. If the undifferentiated blastema cells are capable of redifferentiating into any and all adult tissues, one would have to conclude that the whole of the genotype was still available and that there had been no irreversible inactivation of genes during development. The evidence does not yet go quite so far as this,

since we still do not know the full range of capacities of blastema cells, but it seems to be tending in this direction.

2. *Field action in regeneration*

The process of regeneration usually restores exactly what is missing to complete a normal individual. That means that the growth and differentiation of the material is repeated in the first place to the stump or remaining part of the animal, and in the second to the final complete form. It was this situation, more than any other, which has tempted biologists to employ the concept of 'fields', and regeneration provides the classical context for a discussion of the validity and meaning of this notion. We shall find that so long as it is not taken to provide a solution to the problems, but rather as an enlightening way of formulating them, the field concept can be very useful. Even those cases, known as 'heteromorphoses', in which regeneration does not restore normality, can be usefully discussed in such terms.

There is not space here to discuss regeneration fields in all the groups in which they occur, and we shall limit our attention to certain aspects of the process in coelenterates, platyhelminths and vertebrates. An introduction to the literature, including that concerning other groups, may be found in A. E. Needham (1952).

(*a*) *Coelenterates*

During the eighteenth century, the experiments of Trembley of Geneva on the regeneration of *Hydra* made this topic, for a time, a fashionable diversion in the drawing-rooms of the elegant. Work has continued on it ever since. In recent times, the elongated marine hydroids, such as *Obelia*, *Tubularia*, *Corymorpha*, etc., have been more commonly employed as experimental material (General Reviews: Barth 1940, Child 1941).

The power of regeneration of all these organisms is exceedingly great. The animals consist of an apical region, the hydranth, which is provided with tentacles surrounding the mouth; the main body, containing a gastric cavity; and a foot or stolon region, by which the animal or colony is attached to the substratum and which does not contain any gastric space. The marine forms which have been mainly studied occur as colonies, consisting of many individual polyps united by their gastric portions. These regions are known as the coenosarc, and in many forms are enclosed in a hard chitinous sheath, the perisarc.

Any fragment of the gastric or coenosarc region, which is large enough not to fall to pieces as a result of the wounds inflicted in isolating it, can produce a new hydranth by regeneration; usually it also produces a new

stolon. Most attention has been paid recently to the rate and frequency of the regeneration of the distal end, which gives rise to a new hydranth. This is influenced both by the level from which the fragment is taken, that is the distance behind the original hydranth, and by whether a hydranth remains attached to the fragment or not.

Suppose a piece of *Tubularia* or some similar form is taken, consisting of a hydranth attached to an unbranched length of coenosarc. This is cut in two at some level, i.e. at some definite distance not too far behind the hydranth. The usual result is that a new hydranth is formed at the distal end of the proximal piece, but none appears at the proximal end of the distal fragment to which the hydranth is still attached. If, however, in another similar piece, the cut is made very slightly nearer the hydranth, a similar result occurs; and now the hydranth formed on the proximal piece is arising in the very region which, in the previous experiment, failed to form hydranth. Thus this failure must have been due to its having been still in continuity with the original hydranth, and not to any inherent lack of power of regeneration. This phenomenon, in which the presence of a hydranth suppresses the regeneration of a second hydranth at the other end of a fragment, is spoken of as *dominance* of the hydranth. (There should be no temptation, of course, to confuse this use of the word with that current in genetics.) (Fig. 14.2.)

The dominance of an apical hydranth gradually diminishes along the length of the fragment, and at the proximal end of very long fragments is hardly appreciable. This suggests that the hydranth sets up a high level of some activity, or concentration of some substance, which falls off away from it in a gradient along the coenosarc. Rather surprisingly at first sight, there is no evidence of dominance in very short fragments, which tend to regenerate hydranths on the proximal surface as well as the distal. This can, however, find an explanation if we suppose that dominance only occurs if there is a considerable difference in activity between the two ends of the fragment and that when the isolate is very short, the difference is so small as to be ineffective.

One cannot accurately measure the degree of dominance in longer pieces without taking into account another factor which varies along the length of the coenosarc. Experiment shows that, quite apart from dominance, the inherent rate of regeneration falls off as the distance from the original hydranth increases. Barth (see 1940) investigated this by making a series of equal-sized small isolates from the different levels of the stem. Any phenomenon of dominance within the fragments was prevented by constricting them tightly in the middle by a ligature which effectively isolated the two ends from each other. Both ends then produce hydranths.

The rate of formation of the hydranths falls off from the distal to the proximal fragments, and this gives a measure of the gradient in intrinsic regeneration rate, independent of dominance (Fig. 14.2).

In order to estimate dominance, a fragment of medium length is taken and allowed to regenerate at the proximal end; and its rate of regeneration is compared with that in a similar fragment in which the influence of the original hydranth has been suppressed by a ligature just behind it. Again, if a piece of coenosarc is isolated, the original hydranth being discarded, the distal end will regenerate more rapidly than the proximal, and the

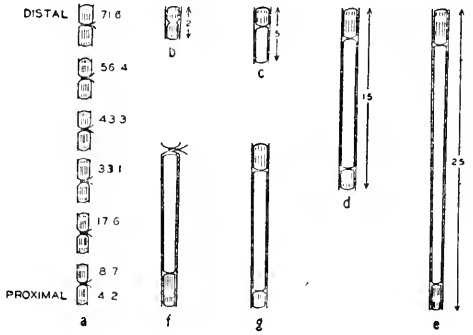


FIGURE 14.2

Figure *a*, rates of regeneration (in μ per hour) at various levels along a stem of *Tubularia;* this is the inherent rate, any interference by dominance being avoided by ligaturing; *b*, *c*, *d*, *e*, showing bipolar regeneration in the shortest lengths, then complete dominance of the distal end, then partial dominance, which weakens in the longest lengths; *f*, regeneration at the proximal end is rapid when a ligature prevents the distal end exerting dominance, but slower (*g*) when dominance is possible. (From Barth 1940.)

distal regenerating hydranth will exert some dominance over the proximal one. The degree of dominance can be measured in the same sort of way as before, by comparing the rate of proximal regeneration when the distal end is left free or is ligatured.

These experiments reveal the existence of two gradients, one of intrinsic regeneration capacity and one of dominance. Since the hydroid stem is a linear structure, we are only confronted with gradients in one dimension, along it. We can take them as being the simplest possible instances of fields, which in other cases will usually extend in two or three dimensions. The two gradients obviously correspond to the two types of fields mentioned on p. 25; the gradient of intrinsic regeneration rate is a 'field' of

competence, that of dominance is an expression of a hydranth individuation field which extends outside the limits of the actual hydranth structure.

It must be clearly recognised that when we speak in this case of gradients or of fields, we are doing no more than describe the phenomena revealed in experiment, and are still far from a satisfying explanation of the mechanisms involved. We can, however, in the case of the hydroids, make some further progress towards this, both in the refinement of the theoretical formulation of the case and in the experimental discovery of new facts about it.

Let us consider the theoretical aspects first. Both Barth (1940) and Spiegelman (1945) have suggested that the mechanism of the individuation field is to be found in a competition between the various regions for physiologically necessary substances. Suppose, for instance, that the production of a new hydranth involves the transformation of some raw material K into hydranth material R. Then clearly if two hydranths are being formed, and are physiologically connected so that they both utilise a common supply of K, the development of one hydranth will tend to inhibit that of the other. If one hydranth gets some sort of start over the other, or is more effective in drawing supplies of K from the pool, then it will be dominant, and, while being little inhibited itself, will have a strong depressant effect on the other.

If one makes assumptions about the character of the reactions, one can put the situation into mathematical language. One of the simplest hypotheses, which is adopted by Spiegelman in his formulation, is that the production of R is (i) proportional to the amount of raw material still available, i.e. to $K - R$; and (ii) the efficiency of utilising the raw material is reduced as time goes on and the concentration of R builds up. The second assumption could be expressed, to a first approximation, by supposing that the rate of formation of R is proportional to $b - cR$, where c is some constant.

For a single process of hydranth formation we should then have an equation of the form

$$\frac{dR}{dt} = (K - R)(b - cR).$$

If there are two competing sites of hydranth formation, we shall have to consider an R, a b and a c for each of them, which we may indicate as R_1, R_2, b_1, b_2, c_1, c_2. Moreover in accordance with the second assumption above, we must expect that the formation of hydranth at one site has an effect on the efficiency of hydranth production not only at that site but at the other one also. We can cater for this by including a new term in the

last bracket of the equation as given above. Thus for two competing hydranths, we shall have to consider equations of the form

$$\frac{dR_1}{dt} = (K - R_1)(b_1 - c_1R_1 - d_{12}R_2)$$

and

$$\frac{dR_2}{dt} = (K - R_2)(b_2 - c_2R_2 - d_{21}R_1).$$

One can see, in a general way, the results which processes of this kind would produce, by considering the situation which would arise when the processes had gone to completion, by which time no further change would be occurring, and the (dR/dt)s would be zero. Then we shall have

$$b_1 - c_1R_1 - d_{12}R_2 = 0,$$

and

$$b_2 - c_2R_2 - d_{21}R_1 = 0.$$

From the first of these equations, we see that, in this final state

$$R_1 = \frac{b_1}{c_1} - \frac{d_{12}R_2}{c_1}$$

Since if there had been no competition (i.e. if d_{12} were zero), R_1 would have been $\frac{b_1}{c_1}$, it is obvious that the competition has led to some degree of inhibition of R_1; and the same is of course true of R_2. Also dominance will occur when either R_1 or R_2 is larger than the other; and this may happen either because of the relations between the intrinsic efficiency constants b or on account of the 'interaction' coefficients c and d, or from certain combinations of these. For instance, if the interaction coefficients are the same for all sites, but there is a gradient in intrinsic efficiencies we shall have $cR_1 = b_1 - dR_2$ and $cR_2 = b_2 - dR_1$, whence it is easy to show that

$$R_1 - R_2 = \frac{b_1 - b_2}{c - d}$$

so that if b_1 is greater than b_2, R_1 will be larger than R_2, and there will be dominance of the site with greater efficiency over that with less. It is clear, without our going into the details of the other possible cases (see Spiegelman 1945), that the assumption of physiological competition does provide a mechanism by which gradients in efficiencies of synthesis or interaction would give rise to phenomena such as dominance. It thus makes it possible to envisage field phenomena in a form in which they become amenable to physiological analysis, aimed for instance at

measuring the relevant efficiencies or discovering what substances are being competed for, etc.

As to the 'substances' involved (we have to use the word in a broad sense), we have two main pieces of information, which have not yet been fully brought into relation with each other. The first to be discovered was that oxygen is highly important. It probably operates in two ways, firstly as a component of the stimulus which sets the regeneration going, and secondly as one of the reactants while the process is proceeding (Barth 1940). Its importance as a stimulus can be demonstrated if a hydranth is cut off and the perisarc pulled forward and ligatured in front of the cut surface, so as to shut it off from the surrounding sea water; no regeneration takes place. The same result can be obtained by covering the cut surface with a piece of glass tube. Moreover, if the perisarc is cut open so as to expose a region of the coenosarc, regeneration may occur, particularly in oxygen-rich water, even if no wound has been inflicted; and the injection of a bubble of oxygen between the perisarc and coenosarc may have the same effect. The continuing importance of oxygen during the whole process of regeneration is shown by the fact that the rate of formation of new hydranth is highly dependent on the amount of oxygen in solution in the water.

Child, and many workers following his lead, have been emphasising for some time the importance of gradients of respiratory rate which they claimed to demonstrate in hydroids and many other regenerating animals, and in eggs in which field phenomena play a leading role (see Child 1941). It was claimed that field processes always depend on gradients in metabolic activity, and that the metabolic activity which is most crucial is that of respiration. The expression 'metabolic activity' is, of course, so general that, in those terms, the hypothesis is little more than a truism; a field must obviously have graded differences between its parts, and the portmanteau phrase 'metabolic activity' could cover these whatever their nature. The part of the theory which it is important to discuss is, therefore, the notion that it is respiration which is basic. Extended discussions will be found in Child's own book (*pro*) and in Needham 1931 (pp. 582 ff.) and Brachet 1945 (*contra*). The general sense of the situation would seem to be that, whereas various indirect methods (e.g. susceptibility to poisons, reaction with vital dyes, etc.) often give evidence for the existence of some sort of gradient, it is by no means clear in most cases that the gradients primarily affect respiration; and moreover it remains obscure whether the gradient of respiration, if there is one, is the causative basis of the observed field or rather merely another expression of it. The two most crucial embryological cases in which critical evidence might be

sought are those of the echinoderm animal-vegetative gradients and the amphibian organiser; in neither of them is a gradient in respiratory rate of demonstrable importance (see pp. 88, 200).

In the hydroids, the situation is somewhat more favourable to Child's ideas, since there is fairly good evidence that a gradient in rate of respiration actually exists. In the early measurements of the oxygen uptake of fragments taken at successive levels behind the hydranth, the experiments lasted so long that an important amount of regeneration would have occurred, and the gradient of rate found, with the hydranth end respiring fastest, may have been mainly an expression of the faster rate of regeneration of this end. But Barth (see 1940) has made accurate measurements over short periods, and finds that there is almost certainly a gradient in this sense in the hydroid immediately after cutting. This, however, does not by any means necessarily imply that the respiratory gradient is a cause, and not a mere concomitant of the gradient in regeneration rate.

Turning now to a type of 'substance' quite other than oxygen, it seems that considerable importance should be attached to the finding of Tardent (1954) that there is a gradient in the concentration of neoblasts or regeneration cells (known also as interstitial cells). This was found both in *Hydra* and *Tubularia* (Fig. 14.3). These cells increase in number near the cut surface, but it is not yet clear to what extent this is due simply to multiplication or how far migration from other parts of the animal is involved. If the latter process is extensive, it may be that they are the most important 'substance' (if one can call them that) for which the two ends of an isolated length of hydroid are competing. It would be very interesting to know whether their rate of respiration is higher than that of the other tissue cells, in which case the respiratory gradient described by Child and Barth might be a reflection of the gradient in neoblasts; but there is as yet no definite evidence on this point.

It seems probable that when a hydranth is removed from a hydroid, the gradient from the new distal end is established in two stages. First there must be an accumulation of neoblasts at the wound surface, presumably as a response to the increased availability of oxygen. But after the initiation of the hypostome or mouth region of the new hydranth, this may be itself responsible for building up the gradient. Several authors have shown that this region, when transplanted into the side of another polyp, is particularly efficient at causing the production of a new hydranth in its neighbourhood, much of which is formed out of tissues of the host (Beadle and Booth 1938, Yao 1945). This is a typical example of an 'assimilative induction', in which the transplant acts as the carrier of a powerful individuation field. In *Hydra*, whose powers of regeneration

are more moderate, only the hypostome can induce a new hydranth in this way. In *Tubularia*, on the other hand, as we have seen, mere laceration, or the injection of a bubble of oxygen, will suffice to produce the same result. In such extremely regenerative forms, the tissues can be cut up finely and reduced to a homogeneous mass, and are still capable of giving rise to a well-organised hydranth from the upper surface where the availability of oxygen is greatest (cf. Barth 1940).

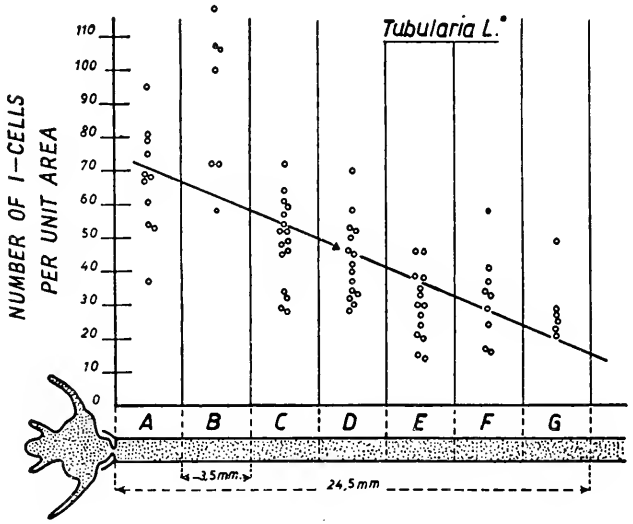


FIGURE 14.3

Frequency of interstitial cells along the stem of *Tubularia*. (After Tardent 1952.)

It is worth noting, as a curious and so far unexplained fact, that a hydranth which is actively in the process of regeneration is not able to restore itself if injured before the process is complete (Davidson and Berrill 1948). The regenerative capacity appears rather suddenly just when the new hydranth reaches its final functional histological state; and it will then not only react by regeneration to any subsequent injury, but will replace parts which have been removed during its development. This may perhaps mean that during the first regeneration all the neoblasts are involved in the process and there are no more available to deal with a second injury, but the matter has not been fully investigated. It is

commonly found that embryos in the most active phases of development have less power of regeneration than at either earlier or later more quiescent periods. Thus in the Amphibia the early gastrula can easily repair defects, and so can the young tadpole, but the neurula or early tail-bud stage has little capacity for regulation.

(*b*) *Flatworms*

It will be useful to supplement the account of regeneration fields in hydroids by a somewhat shorter discussion of similar phenomena in flat worms, which are also a lowly group of invertebrates but somewhat more highly evolved than the coelenterates (General Reviews: Brønsted 1954*a*, *b*, Wolff 1953). We shall find that in the flatworms the active individuation field in the adult is less powerful than that which is maintained by the hydranths in hydroids. As a consequence of this the main role in directing the early course of regeneration is played not by the adult organs which remain in the regenerating piece, but rather by a static field of regeneration potential, which can be compared with the gradient in regeneration rate which was characteristic of the hydroid stem. This determines the character of the blastema which is formed, and that in its turn then brings about the development of the appropriate organs.

Regeneration has been mainly studied in triclads, of which *Planaria* is a characteristic genus. The whole group are frequently referred to as planarians. If a planarian is cut in two by a transverse cut it is frequently found that the anterior segment regenerates a tail and the posterior segment a head. In both cases the process starts by the formation of a blastema, which is produced by neoblasts which migrate to the wound surface (see p. 306). Many species of planarians are provided with eyes in the anterior region of the head. One of the first signs of head regeneration is the appearance of such eyes in the blastema. This occurs at an early stage, even if a large part of the anterior of the worm has been removed. Thus it seems that in regeneration of the head the most anterior part is formed first and whatever else is required is, as it were, intercalated between this anterior part of the head and the remaining posterior end of the body. During the later stages of regeneration, however, a good deal of morphallaxis occurs, that is to say a remodelling of the original posterior part of the body.

The occurrence of these easily recognised organs, such as eyes, makes it simpler to study the regeneration of the head than that of the tail, and most work has concerned itself with this type of regeneration. The simplest experiment consists in placing the transverse cut, which divides the worm in two, at various levels from the anterior to the posterior. We find

that, as was the case in the hydroids, any particular level of the body may form either a head or a tail according to whether it is attached to an posterior or to a anterior piece. However, the ability to regenerate a head, as measured either by the frequency with which this is successfully accomplished or by the time taken to do so, is not usually constant along the whole length of the worm. Different species fall into a number of classes in this respect. There are some in which no regeneration of a head occurs from any part; in others, of which the well-known *Dendrocoelum lacteum* is one, the anterior end regenerates easily but the ability to form a head falls off rapidly and has disappeared at the level of the pharynx. In others, the curve expressing the ability to regenerate a head (the so-called 'head-frequency curve') falls off more gradually and even the most posterior end of the body sometimes carries out the regeneration successfully. Some *Planaria* normally reproduce by transverse fission, a new animal forming in the posterior end of the body of an old one. In these the head-frequency curve, after falling in the region of the pharynx, rises again towards the posterior end. Finally there are some species, such as *Planaria velata*, in which the ability to regenerate a head appears to be equal along the whole length of the body. There are also tail-frequency curves in all these species, but less is known about them.

The ability to regenerate a head is, however, not fully expressed merely by a curve which assigns some definite ability to each body level. There is, in point of fact, a gradient from the midline of the body towards the margins as well as from anterior to posterior (Fig. 14.4). We have to deal with a two-dimensional field of head-forming ability rather than with a one-dimensional gradient of it. The type of regeneration which occurs when the planarian body is cut in more complicated ways can usually be deduced fairly simply from the principle that in posterior pieces a head forms at that point of the cut surface which has the highest value in the head-producing field. To account for the fact that the same point would form a tail if attached to the anterior piece, the simplest assumption seems to be that the regeneration tends to occur in such a way as to carry on the gradient which is already intrinsically present within the fragment. In this way one can understand such peculiar phenomena as the appearance of the two heads at the shoulders of the T-shaped cut shown in Fig. 14.5A-D. The appearance of heads in the situation shown in Figs. 14.5E and F is not so fully accounted for, since here the regenerating edge is connected directly both with the anterior and the posterior parts of the body, and further subsidiary hypotheses would be necessary before it was clear what we might expect to obtain. There seems at present to be no adequately tested hypothesis which can deal satisfactorily with all the

numerous and complicated experiments which have been made in this field.

It is important to note that it is only regions of the old body in the immediate neighbourhood of the cut which exert an influence on the course of regeneration. Brønsted (1939) has isolated a section of the body anterior to the pharynx by two transverse cuts (in *Euplanaria lugubris*) and then grafted the anterior tip containing the original head with reversed polarity to the posterior edge of the isolated segment. The grafted head in this

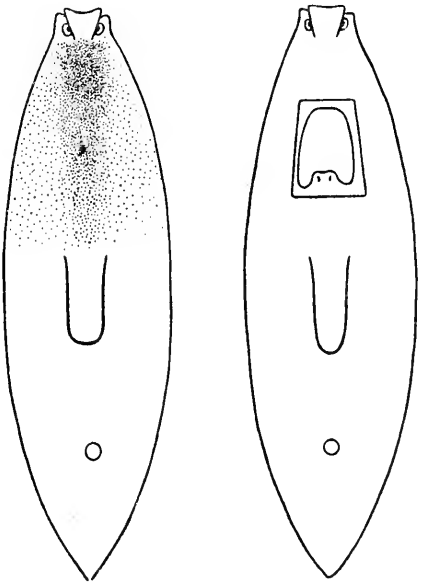


FIGURE 14.4
On the left, the head-regeneration field in the planarian *Bdellocephala punctata*. On the right, a head regenerated in a 'window' cut in the anterior region. (From Brønsted 1946.)

situation exerted no apparent influence on the regeneration at the anterior-cut surface, which proceeded to form a head exactly as it would have done normally. Similarly, if a window is cut in a planarian, as in Fig. 14.4, the existing old head does not inhibit the regeneration of the new head in the window. Again, Raven and Mighorst (1948) have shown that the rate of head formation at a given level in *Euplanaria lugubris* is not in the slightest affected by the presence or absence of a further posterior cut at which, if it is present, a tail will simultaneously be regenerating. Thus in planarians there is little or no evidence of competitive interaction between the two ends of an isolated segment, or between a regenerating surface and

already existing organs. We have thus little sign of anything corresponding to the phenomenon of dominance in hydroid regeneration.

There are, however, some inductive interactions in operation in planarian regeneration. If the head region of *Dugesia lugubris* is grafted into the posterior of the body, behind the pharynx, it can induce the formation of a supernumerary pharyngeal region from its neighbourhood; while in *Polycelis nigra*, the cerebral ganglion induces the formation of eyes

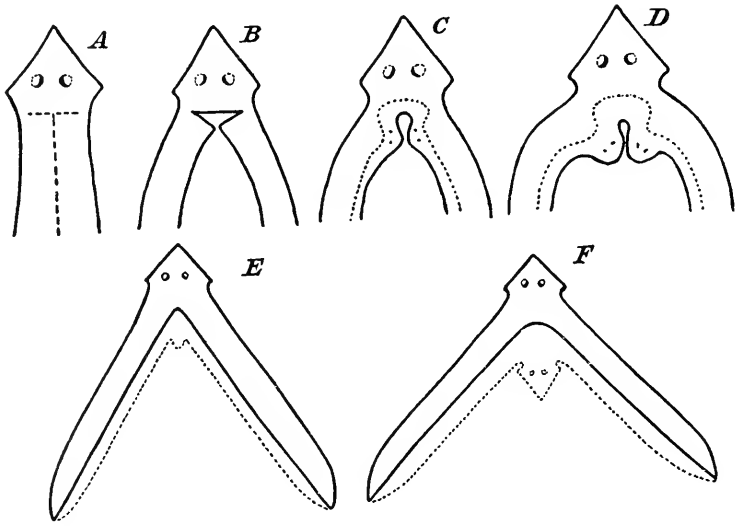


FIGURE 14.5

A, B, C, D, stages in the regeneration of two heads, following the T-shaped cut shown in *A*; *E, F*, regeneration of a head following a longitudinal cut. (After Beissenhirtz, from Brønsted 1946.)

though only from a limited competent area which extends from the anterior to the level of the pharynx (work of Sengel and Lender, see review of Wolff 1953). Wolff has summarised the conclusions from these experiments in a diagram which is reproduced as Fig. 14.6. The first event in a blastema engaged in head regeneration is the formation of the ganglion; this induces a cephalic region (which in many forms includes eyes); this region induces a pre-pharyngeal region, and that again a pharyngeal region; and in the latter the pharynx itself eventually appears. These inductive relations can be regarded as the expressions of an individuation field which is most powerful when it arises within a blastema, since it can then affect even the anterior part of the body, but which persists at a lower intensity even in the fully developed organism.

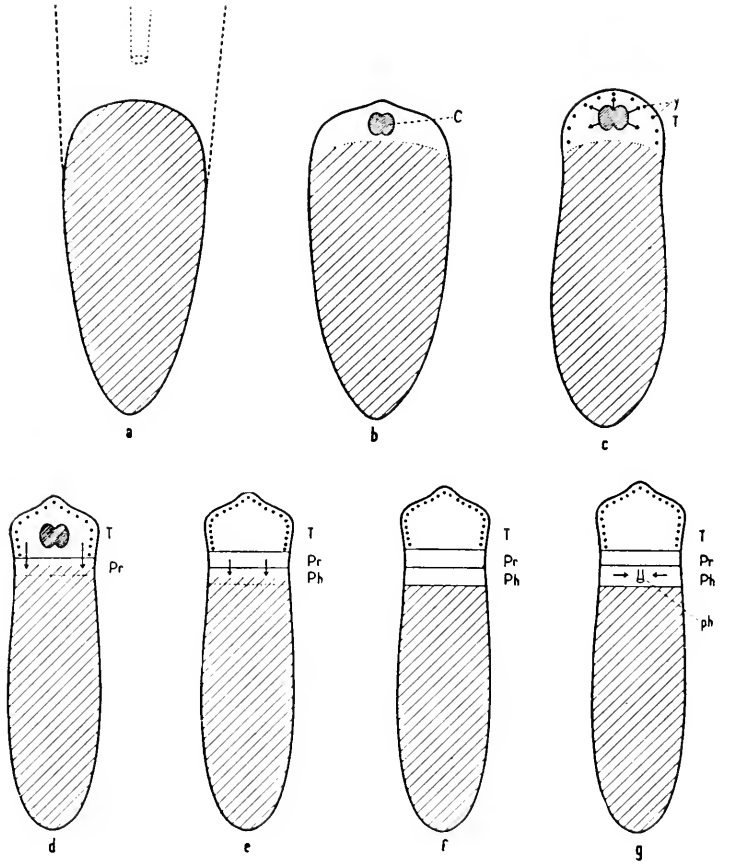


FIGURE 14.6

Successive inductions during the regeneration of planarians. After a posterior region is isolated (*a*) the first step is the formation of a ganglion (C) in the blastema (*b*). This induces a head (*T*) provided with eyes (*y*). The head region then (*d*) induces a pre-pharyngeal zone (Pr), and that in turn (*e*, *f*) a pharyngeal zone (Ph), in which (*g*) a pharynx finally appears. (From Wolff 1953.)

(*c*) *Amphibia*

Larval and adult urodele Amphibia (newts, salamanders, axolotls, etc.) can regenerate legs or tails and other organs fairly readily (General Reviews: J. Needham 1942, A. E. Needham 1952). The capacity of Anura (frogs, toads, etc.) is much less in this respect, the power of regeneration usually being lost at about the time of metamorphosis. The regenerative phenomena in urodeles provide a very good example of one kind of field action. It was in fact in this connection that the concept was first extensively discussed by Weiss, one of those who introduced the notion of

fields into embryology, and who contributed greatly to the early experimental work on vertebrate regeneration (see Weiss 1930, 1939). As we shall see, however, the field which is operative in amphibian regeneration is strictly an individuation field concerned with the building up of a complete unit, and shows no sign of activity outside the limits of this unit. There is therefore nothing which corresponds to the dominance of the old hydranth in the regeneration of hydroids.

As far as regeneration is concerned the urodele body can be considered to be made up of a series of organ-districts. If a complete district is removed, it cannot be regenerated. If, however, any part of the district is eliminated regeneration will restore what is missing, unless indeed for some reason the blastema is smaller than normal, when a deficient organ may be produced. The limit of the organ district of the tail, for instance, is the last sacral vertebra. If the tail is amputated anterior to this, no regeneration occurs, whereas if the cut is made anywhere further posteriorly, a complete tail is formed. Regeneration occurs strictly within each organ district and is uninfluenced by the position of that district within the body as a whole. Thus if a limb is transplanted to the middle of the back and then amputated, the stump nevertheless regenerates a limb. The character of the regenerate, in fact, depends on the tissue in the immediate neighbourhood of the wound. If a hindlimb is amputated, a forelimb transplanted to the stump, and then the forelimb again amputated so as to leave only a small section of it, the regenerate will still be a forelimb and show no influence of the hindlimb stump, which is further away from the wound surface.

Amphibian regeneration exhibits several peculiar polarity phenomena. In the first place it should be noted that the regenerate always produces the parts distal to the wound surface and apparently never those proximal to it. Thus if a deep V-shaped cut is made into a limb, both faces of the wound may produce a regenerate and both of these will develop into the regions of the limb which should lie distal to the cut. Again, if a segment of a limb, say the region near the knee joint, is isolated by two cuts and then inserted into the body-wall in such a way that the proximal surface as well as the distal can regenerate, it will be found that the proximal surface does not form a new femur and upper part of the limb, but that both surfaces form the distal extremity. J. Needham (1942) accepted some earlier data which suggested that in such cases the proximal surface regenerated only so much of the lower limb as had originally been isolated. This would have been a very peculiar situation, since it would have meant that the individuation field had been permanently altered by the isolation. It appears, however, from more recent work of Monroy

(1942), that the regenerate from the proximal surface forms the whole missing region and not only a part of it (Fig. 14.7). We can conclude therefore that regeneration will always produce the whole missing region from the cut surface to the distal tip of the organ, even if this means that a reversal of polarity has to occur at the place where the cut was made. It may be pointed out that if a section of a limb is removed and the distal region grafted back on to the stump, no regeneration of the missing segment occurs, probably because there is no opportunity for a blastema to form.

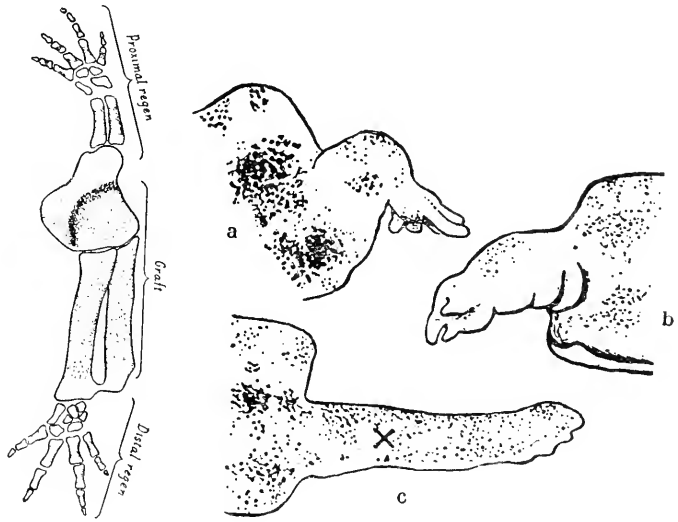


FIGURE 14.7

(Left) Part of an anterior limb of a newt, consisting of sections of the humerus, and of the radius and ulna, was grafted into the flank. Both ends have regenerated and each blastema has produced all the structures distal to the position of the wound from which it arose. (From Monroy 1942.) (Right) Two limb-like regenerates (*a* and *b*), and one (*c*) showing features of both tail and limb, all from axolotl tails onto which limb-skin has been transplanted. (From Luther 1948.)

Regeneration, of the limb at least, is not only limited by the fact that it proceeds only distally and never proximally: it can also not proceed laterally. That is to say, if a limb is split longitudinally and one half removed, this half is not replaced. However, if the half-limb is now amputated regeneration occurs and the regenerated portion of the limb is complete in cross-section. Similarly, if one of the long bones of a limb is removed, it will not be regenerated, but if the limb is now amputated

through the boneless region, the regenerate will be provided with the full necessary complement of bones (the rule does not hold for the tail, see page 308). This formation by the regenerate of structures which are more complete than the stump shows clearly that regeneration is not merely a result of the outgrowth of the tissues of the stump, but is a phenomenon in which an individuation field arises in the blastema and leads to the development of the complete section of the organ between the level of the cut and the distal tip. However, although the structure of the regenerate is not directly produced by the outgrowth of the tissues exposed at the cut, those tissues are operative in determining the character of the individuation field which arises. In most cases, as we have seen, a mere absence of a tissue does not lead to any deficiency in the individuation field. If, however, tissues from two different organs are mixed, compound or hybrid individuation fields may appear. Thus Liosner and Woronzowa (1937) and Monroy and Oddo (1943) have found that if muscles from the tail are grafted into the urodele limb at the site of amputation the regenerate may be intermediate in structure between a tail and a limb. Similarly intermediate structures were found by Luther (1948) after the transplantation of skin from the foot to the tail (Fig. 14.7).

SUGGESTED READING

For a full treatment, A. E. Needham 1952. Other recommended reading: Weiss 1939, pp. 458–478; Barth 1940, Rose 1948*b*; Avel 1940 (oligochaetes, not treated in the text); Spiegelman 1945.

PART TWO

THE FUNDAMENTAL MECHANISMS OF DEVELOPMENT

IN the introductory chapter a list was given of the basic concepts which experimental embryology has developed—those of determination and differentiation, brought about by the mechanisms of ooplasmic segregation, evocation and field action. The accounts of the development of the various classes of animals given in Part One have exhibited the type of facts from which these concepts have been derived. The facts have, for the most part, been obtained by typically biological modes of experiment; for instance, by the transplantation from one place to another of whole cells, or even large masses of cells. The theories to which they have given rise have in consequence also been framed in terms of concepts which belong to the biological realm and cannot be immediately brought into line with the ideas of the more fully developed sciences of physics and chemistry. As Weiss (1947, 1950*b*) in particular has emphasised, we cannot be permanently satisfied with this situation, but must attempt to push our analyses down towards the level at which we are discussing the interactions, combinations and synthetic activities of particular substances. In this connection he has coined the expressive phrase 'molecular ecology' and there is no doubt that a fully developed embryology ought to be able to expound the processes of development in terms of the changes in the populations of molecules making up the cells of the different tissues and should not have to rely on concepts such as evocation and competence, which are special to it alone.

The programme which Weiss proposes is, however, a very difficult one and we are still far from the goal which he envisages. Indeed, the distance is so great that there is probably some danger in attempting to cover it in a single step. There is an intermediate level, between that of the organisers, fields, etc. usually considered by embryologists and the ultimate molecular constituents of living matter, which requires to be thoroughly explored before we can feel the ground firm enough under out feet to have any confidence in attempting to frame theories in chemical or physical terms. This is the level which deals with the activities of the different categories of cell constituents. As we have pointed out, experimental embryology has already approached it in several different contexts; for instance, in relation to the cell granules of mosaic eggs (p. 101), the mitochondria of sea-urchins (p. 90) and the microsomes of Amphibia (p. 212). These are all constituents of the cell cytoplasm, not of the nucleus. The crucial role of inductive processes (both between tissues

and within embryonic fields) demonstrates that the initial differentials which guide the development of various parts of the egg into different channels depend in many cases on substances which can pass from cell to cell and which must therefore be extra-nuclear. There is another method of approach which has penetrated very deeply into this field of the activities of cell parts, that is the science of genetics which has operated mainly by the study of heredity. The knowledge it has yielded has in the main concerned the effectiveness of the nucleus and the chromosomes, an aspect of cell behaviour about which the methods of experimental embryology have not told us very much. More recently, moreover, genetics has begun to produce most valuable information about cytoplasmic particles, which it knows under the name of 'plasmagenes'.

It would, of course, be inappropriate to attempt to give even a sketch of all aspects of genetics in a book devoted to embryology, but there is great deal of genetical information which is not merely relevant but which is essential to our purpose. In fact, when we attempt to discuss the fundamental mechanisms of development we are in a region in which the distinction between the sciences of genetics and embryology breaks down. Genetics using its normal method of studying heredity has revealed the existence of genes which control to a large extent the character of the animal which will develop from the fertilised egg; the way in which the genes operate in doing this belongs by definition to embryology. The union of the two sciences could hardly be more intimate.

In the first chapter of this part we shall discuss in broad outline what genetics has revealed about the general nature of the epigenetic system and the way genes are involved in it. This supplements the experimental embryological material, to complete the picture of development at the biological level. We shall then pass on to consider the basic mechanisms of development in terms of the activities of cell constituents, using facts some of which are conventionally considered to belong to genetics, others to embryology. The greater part of this discussion will be concerned with the differentiation of substance which occurs during development; that is to say with the formation of new materials or new types of tissue. The last chapter deals with the other aspect of development, the moulding of tissues into organs of definite shape and form.

CHAPTER XV

THE ROLE OF GENES IN THE EPIGENETIC SYSTEM

THE SCIENCE of genetics has clearly shown that when an animal differs from nearly related forms, the nature of these differences is nearly always controlled by genes carried on its chromosomes. It is clear then that genes must be amongst the most important causal entities which play a role in guiding development. We have so far discussed the question of why an organ, such as a limb, develops as it does in terms such as organisers, fields, competence, etc. Genetics, following a quite different mode of analysis, formulates its answer to the same question in a quite different way. It finds that the development of the organ is dependent on the activities of certain genes in the fertilised egg. The task of this chapter is to present the picture of the development of an organ or tissue as seen in terms of genes. This will provide a view of the epigenetic system which we must take as being complementary to that derived from experimental embryology.

1. *Developmental pathways and their genetic control*

In using the data of genetics to throw light on the general character of developmental processes we are not concerned with the way in which any particular gene obtains its effect—a question we shall take up in the next chapter. Here we want to start from the other end, taking an organ or tissue and seeing how genetics would lead us to envisage its development. It will be convenient before discussing particular cases to summarise the general principles which we shall in fact find to emerge. The most important of these are:

(1) The development of an organ or complex substance takes place in a series of steps, each of which is affected by genes.

(2) At each step there are several genes acting, and the actual development which occurs is the resultant of a balance between the opposing gene-instigated tendencies.

(3) At certain stages in the development of an organ, the system is in a more than usually unstable condition, and slight disturbances at such times may produce large effects on the later events. Such times have been called 'epigenetic crises'.

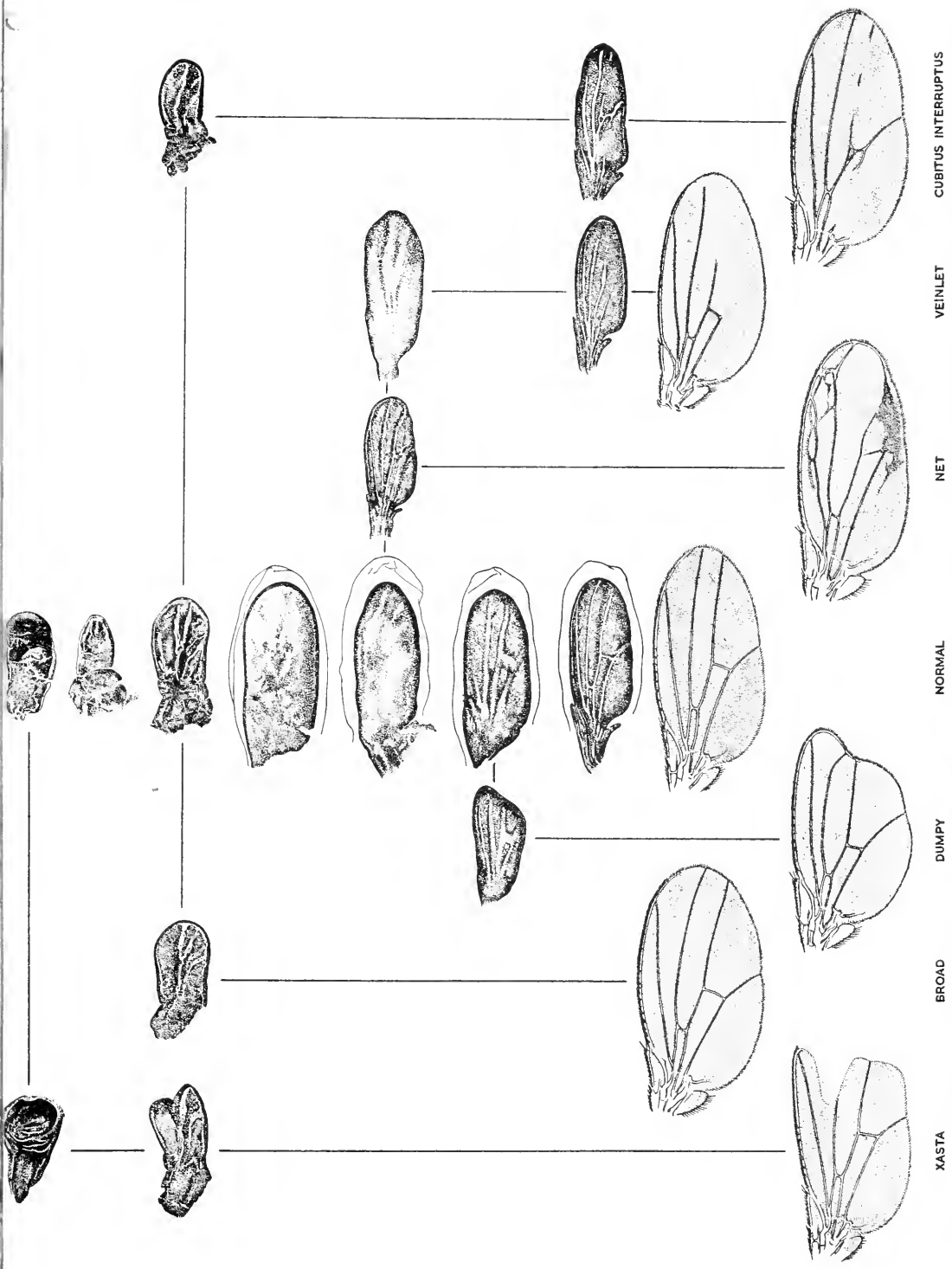
(4) An organ or tissue is formed by a sequence of changes which can be called the 'epigenetic path' leading to it. In a normal egg which contains the genes usually found in the wild individuals of the species, these paths are rather definitely distinct from one another, so that a developing

mass of tissue turns either into a leg or into a wing, say, but it is difficult to persuade it to become something intermediate. And also each path is 'canalised', or protected by threshold reactions, so that if the development is mildly disturbed it nevertheless tends to regulate back to the normal end-result (Waddington 1940*a*).

As a first example in which these principles may be seen, we may consider the development of the wing in *Drosophila* (Goldschmidt, 1935*b*, 1937, Waddington 1940*b*). The main features are shown in Fig. 15.1 in the form of somewhat diagrammatised drawings of the whole wing at various stages during its development in the pupa. In the larva at the time of puparium formation, the wing is a thickened area of the dorsal mesothoracic buds, an area which is already folded in towards the interior of the bud. Very shortly afterwards, the fold elongates and breaks through the thin opposite side of the bud (stage 2). The thick but pointed blade expands in area and becomes thinner, and as it does so, it becomes apparent that there are channels left open between the upper and lower surfaces; these are the pre-pupal veins (stage 3). Soon the wing, as well as expanding in area, becomes fatter by an inflation which forces the two surfaces apart, obliterating the venation until the wing is transformed into a featureless sac (stage 4). The greatest swelling is reached just about the time of true pupation. From then onwards, the wing starts to contract again. As the two surfaces come together, they leave spaces between them: these are the adult veins, which appear first near the tip of the wing, and gradually spread back towards its root (stages 5, 6, 7). At first the tissue between the veins is spongy and loose in texture, but gradually it becomes more compact, the fluid which had filled the inflated wing sac being finally driven out to give an immature wing almost identical in outline with the final adult one, though smaller than it in size. In fact, after the last pupal stage drawn in the figure nothing much happens to change the morphology of the wing except the expansion of the cells, throwing the whole structure

FIGURE 15.1

The centre column shows eight stages in the development of the *Drosophila* wing during the pupal period, from the condition of an imaginal bud at the top, through the period of inflation to the adult condition. On the left are three gene-controlled modifications affecting wing shape: *Xasta* produces a distal nick as early as the imaginal bud stage; *broad* affects the direction of growth in the prepupal stage before the inflation; *dumpy* increases the longitudinal contraction after the inflation. On the right are three genes altering wing venation. *Cubitus interruptus* removes one of the prepupal veins. *Net* causes the appearance of extra venation, and *veinlet* the obliteration of the tips of the normal veins, during the contraction following the inflation. (Adult wings to smaller scale; after Waddington 1940*b*.)


CUBITUS INTERRUPTUS
VEINLET
NET
NORMAL
DUMPY
BROAD
XASTA

into folds which only become flattened out again after the imago emerges from the puparium.

The final wing is a simple-enough organ. It is practically two dimensional, since the thin upper and lower surfaces fuse tightly together; its outline is a simple oval slightly indented where the most posterior vein cuts it; and the whole system of venation consists only of one vein forming the fore-edge and four main longitudinal ones radiating from the base of the wing with two short cross-veins between them.

The wing path of development is affected by very many genes; Waddington (1940*b*) has described the abnormalities produced by some thirty of them, and a fair number of others are known. The mutant alleles of these genes are recessive to the wild-type alleles; that is to say, the epigenetic path is canalised to the extent that an alteration of only one of the two alleles to the mutant form does not suffice to produce any noticeable alteration in the course of development, presumably because some threshold is not exceeded in the heterozygote.

A few of the recessive forms are illustrated in Fig. 15.1, which shows how their development diverges from the normal pathway. Each step in the normal sequence is influenced by genes, which often act upon the developing tissues in opposite directions. For instance, in the prepupal wing, the relative rates of cell division in different directions are affected by the genes *broad*, *expanded*, *lanceolate* and *narrow*, of which the first two cause the wing to become broader and the last two longer. Again, the time of the pupal contraction is a minor epigenetic crisis, during which the contracting wing is in a state of delicate balance, influenced by genes such as *dumpy*, *humpy* and *spade*, which tend to increase the contraction in length, *blade* which tends to increase it in width, *balloon* and *bloated* which tend to reduce the contraction in general; while genes whose primary effects are to change the shape of the wing margin may produce secondary effects at this time, since if the wing is abnormally long and narrow, or short and broad, before the contraction starts, these characteristics will become exaggerated. During the later stages of the contraction, the imaginal veins appear as cavities remaining between the two wing epithelia and there are some genes, such as *veinlet*, *tilt*, *radius incompletus*, which tend to cause obliteration of veins, while others, such as *plexus* or *net*, work in the opposite sense, and produce extra veins.

From a study of this large number of genes affecting the development of a single organ, a picture of the general epigenetic situation emerges. Direct investigation by more conventional experimental techniques has confirmed it in many points. Thus Lees (1941) made defects in the developing wing by pricking it with a needle at various definite times, and could

interpret his results in terms of the same sequence of developmental mechanisms as the genetic study had revealed. Goldschmidt (1935*a*) Henke (1947), Schatz (1951) and others have treated *Drosophila* pupae with just sub-lethal temperatures at definite times, and found that a series of abnormalities of wing development occur which parallel in a striking manner the forms produced by mutant genes. Such abnormalities which are similar to the phenotypes characterising genetic races but which are produced by environmental stimuli, are known as '*phenocopies*'. Henke found the interesting fact that the time at which a temperature shock was capable of causing the appearance of a phenocopy of some particular mutant type was usually near to, or just before, the time at which the development of that type first diverges visible from the normal. For instance, shocks just before or during the contraction phase tend to produce *dumpy*- or *blade*-like phenocopies. For each type of phenocopy there is a 'sensitive period' during which it can be relatively easily induced[1] (Fig. 15.2).

The fact that the sensitive period for the phenocopy of a gene occurs very near the time at which the development of the mutant becomes recognisably abnormal suggests at first sight that this is the stage of development at which the gene becomes active. It is, however, by no means clear just what might be meant by 'the time of action of a gene'. If an embryo contains a certain mutant gene, that gene is present in its cells from the time of fertilisation onwards. Long before there is any visible abnormality of development, the gene may have been producing some unusual substance which is merely stored up within the cell without being detectable by existing methods. The fact that an external agent can produce a given phenocopy most easily at a certain stage of development is a sign that the relevant epigenetic process is most unstable at that time. It is not surprising that it should be just at this time that the mutant gene also begins to produce detectable divergences from the normal course of development, but this does not tell us whether the gene has been active earlier or not. Nor, of course, are we justified in concluding that the gene acts by a mechanism similar to that of the environmental stimulus; in fact during a sensitive period many different external stimuli (heat, cold, x-rays, etc.) may produce similar effects, and it is clearly impossible that all of them can be disturbing the system in the same way that the gene does. Phenocopy studies can thus provide some information about the stability of the epigenetic situation in the developing tissues, and can

[1] Occasionally there are two different sensitive periods, for instance when very similar end-results can be brought about in two different ways.

reveal the 'point of attack' of a gene, but can as a rule tell us little about the gene's mode of operation or the time of its primary activity.

The sensitivity of a stock of animals to environmental stimuli is under genetic control. One can, by selection over a number of generations,

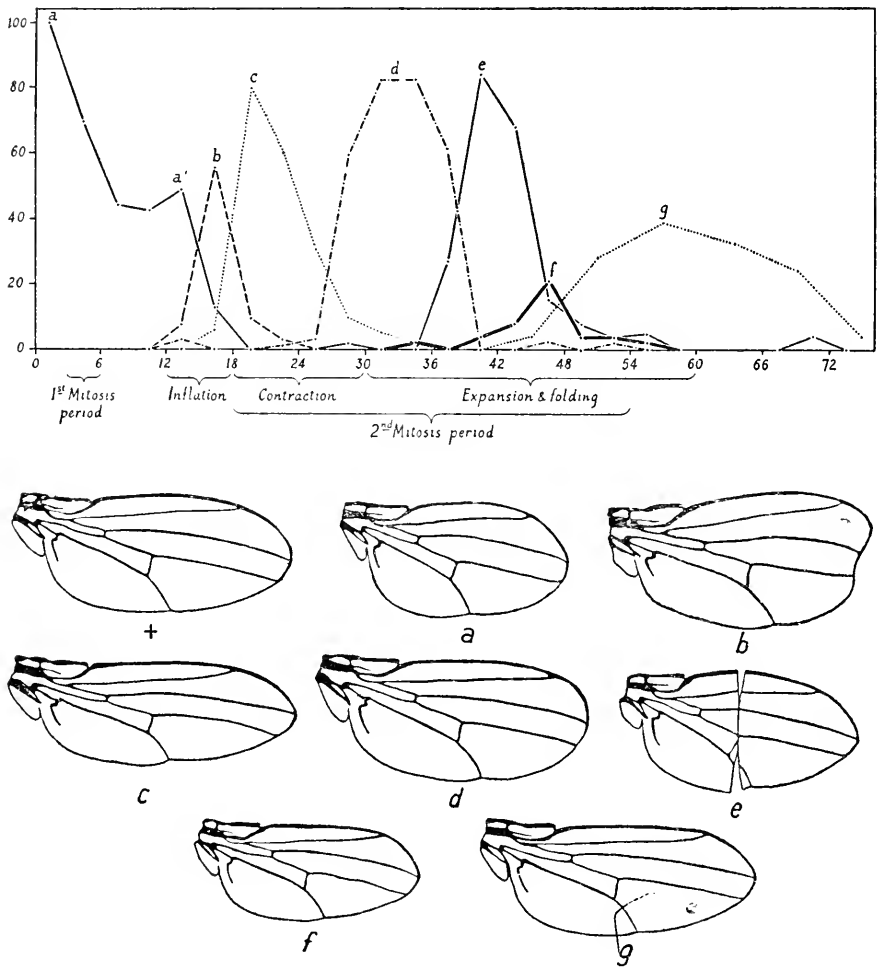


FIGURE 15.2

The curves above show the frequencies per cent of various types of phenocopies produced when a temperature shock (3–5 hrs. at 39° C.) was given at certain times after puparium-formation to a stock of *D. melanogaster*. The type of wings corresponding to the various curves are shown below, together with the wild type (+). They are: (*a*) first broadened type; (*b*) dumpy type; (*c*) first narrowed type; (*d*) rounded end type; (*e*) second broadened type, usually curved as well; (*f*) normal proportioned but small; (*g*) second narrowed type. (From Schatz 1951.)

build up strains whose hereditary constitution predisposes them to respond in particular ways to any given treatments. This specification of the developmental stability of the organism is surprisingly precise and detailed; it usually depends, in any given case, on rather a large number of genes; and starting from any fairly large population, it seems to be always possible to find in it genes which will confer on their bearer almost any type of developmental reactivity one chooses. These facts again bring to our attention very forcibly the complexity of the genetic system which controls developmental processes. (For discussion of the evolutionary implications of phenocopy-formation and similar phenomena, see Waddington 1953*b*, 1954*b*.)

We may now turn to another example of a developmental sequence which has been well analysed from a genetical point of view (Lees and Waddington 1942, Lees and Picken 1945). The formation of the bristles (macrochaetae) in *Drosophila* appears, by histological investigation, to be an extremely simple process, directly involving only two cells per bristle[1] (Fig. 15.3). In the pupae of about fifteen hours' age slightly enlarged cells may be found in the hypodermis. Already they are sometimes in pairs, though they may also occur singly. By nineteen hours they are always paired, and it is probable that a division, producing a pair of cells, occurs in the period from about twelve to eighteen hours. The two cells proceed to grow rapidly, attaining a volume about a thousand times as great as that of the neighbouring hypodermal cells. During this process the nuclei enlarge greatly, and the chromosomes assume the polytenic banded form which is best developed in the salivary gland cells. The pair of cells become arranged in a characteristic way, with one lying above and slightly to one side of the other. The upper cell, which is known as the tormogen, is destined to form a more or less circular socket of hardened chitinous material, while the lower one, the trichogen, produces a long gradually tapering bristle which sticks up through the centre of the socket. A whole series of genes affects this relatively simple sequence of processes. In the first place there are some, such as *scute*, which cause an absence of certain of the normal bristle cells, and others, such as *hairy*, which produce extra pairs; nothing further is known about their mode of action. The next stage, that of the cell division to produce the trichogen-tormogen pair, is affected by *split*, which often provokes an extra division to give a group of four cells; these become somewhat irregularly arranged, and may give rise to two trichogens and two tormogens or only one of the

[1] There are one or more other pairs of cells closely associated with these; a general description is given by Henke (1953).

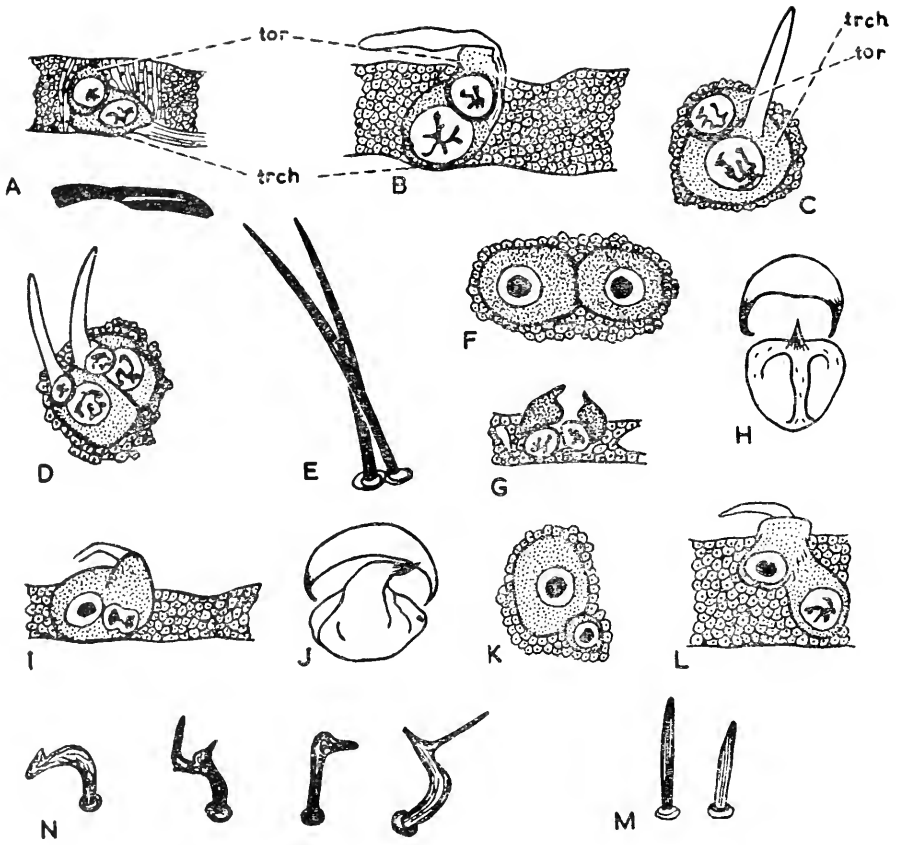


FIGURE 15.3

The genetic control of bristle development in *Drosophila*. *A* and *B* are sections through the thoracic hypodermis of the mid-pupa, showing two stages in the development of the bristle-forming (trichogen, *trch*) and socket-forming (tormogen, *tor*) cells. *C*, surface view. *D*, surface view showing the extra division which occurs in *split*, giving (*E*) duplicated bristles. *F*, surface view, and *G*, section, in *Hairless*, when the trichogen and tormogen lie side by side at the same level, and give two sockets (*H*). In *shaven-naked* (*I*, *J*,) the situation is similar but usually not so extreme. In *Stubble* (*K*, *L*, *M*) the tormogen does not embrace the trichogen as closely as usual, and an abnormal thick bristle is produced. *N* shows the irregular bristles produced by *forked*. (From Lees and Waddington 1942.)

former and three of the latter. Another gene, *dichaete*, has a somewhat similar action, but it is usually only the tormogen which divides a second time and becomes doubled. In the next step, the precise arrangement of the tormogen above and to one side of the trichogen is also under genetic

control. In *hairless*, *shaven-naked* and *prickly*, both cells lie side by side at the surface and both produce sockets, so that no hair is formed; while in *stubble* the tormogen is shifted slightly to one side and sits less firmly on the trichogen, and the hair is thicker and shorter than normal. These phenomena indicate that the shape of the bristle is at least partly due to the moulding of the still-fluid secretion of the trichogen as it is forced through the constricting ring of the tormogen. Several genes affect the nature of this secretion and the rate of its production. *Spineless* and *morula* reduce its amount considerably, so that only short and thin bristles are formed, and the secretion of the trichogen is also reduced to the level characteristic of the tormogen in those mutants in which both cells lie side by side at the surface (*hairless*, *shaven-naked*, *prickly*). In *stubble* the early rate of production is increased but the final value is much the same as in normal animals. Some other genes have a more subtle effect. During its secretion the bristle seems to consist of a thin plastic wall and a more fluid core, the whole of which eventually hardens and becomes hollow. The wall is probably formed of an oriented long-chain high-polymer in a rubber-like state, almost devoid of cross-linkages between the chains. It normally grows just at the rate required to provide enough surface to accommodate the fluid secretion which is being forced into it by the trichogen. This delicate balance between growth in surface and in volume is disturbed by certain mutant genes. In *singed* and *forked* the volume may suddenly increase too fast (or the surface too slowly) so that the bristle 'explodes' and forms bulges and kinks, while in *bristle* there are gradual and periodic changes in the volume surface/relationship, so that a series of fairly gentle swellings arises. Moreover these genes also effect the mechanical properties of the surface, which is more plastic in *singed* and *forked* and more elastic in *bristle*.

Many of these mutant types of bristle may also be produced in phenocopies, but the correlation between the sensitive periods and the points of attack of the genes is not so fully worked out as for the wings.

An even more impressive demonstration of the real complexity of apparently simple developmental processes is provided by the chemical investigations which have been made in recent years on the eye pigments of *Drosophila* (Beadle 1945, Ephrussi 1942, Nolte 1952) and various other insects, e.g. the meal worm *Ephestia* (Kühn 1941, Caspari 1949) and the silkworm *Bombyx* (Kikkawa 1953). In *Drosophila* at least twenty-five genes are known to affect the colour of the eyes, which normally are a slightly brownish red. Analysis of the effects produced when two different mutant genes are both present in homozygous condition led to the suggestion that there are two main pigments, a brown and a red,

and these were later found to be separately extractable with suitable solvents. They are both of relatively low molecular weight, though probably bound to proteins when they are in the natural state in the living cell.

Rather little is known about the development of the red pigment, except that it fails altogether in the absence of the normal allele of *brown* (bw^+) and is affected less profoundly by many different genes. Chromatographic analysis has quite recently shown that it is in fact really a mixture of a rather large number (about ten) of different components (Heymann, Chan and Clancy 1950) and that certain other fluorescent components are closely associated with it (Hadorn 1951*a*, Hadorn and Mitchell 1951).

The production of the brown pigment provides a good example of a sequence of developmental steps, and is particularly interesting because it has been possible to discover the actual chemical changes involved in some of these. Sturtevant (1932) pointed out that in a fly heterozygous for vermilion (v/v^+) it can sometimes happen that the v^+ chromosome gets lost at a mitosis, and that a patch of cells with the constitution v may appear among the heterozygous eye facets, which are normal in colour. When this happens with most other genes, an abnormally coloured group of cells can be clearly seen, but no departure from the normal wild-type pigmentation is found in patches which can be shown, on other evidence, to be v in constitution. Sturtevant therefore suggested that some substance diffuses into these cells from the surrounding tissue and compensates for the absence of the v^+ gene.

Beadle and Ephrussi developed a technique of transplanting pupal eye-discs into the body-cavity of other larvae, and by suitably choosing the genotype of the host and of the transplant, were able to show that there are at least two diffusable substances (which they rather unhappily called 'hormones') concerned in the production of the brown pigment. One of these is produced under the influence of the normal vermillion gene (v^+) and is lacking if that gene is replaced by the mutant-vermillion allele (v), while the other is similarly related to the cinnabar gene. Investigation finally showed that these substances are derived from tryptophane, which is converted first (under the action of v^+) into α-oxytryptophane, which is then oxidised (by a reaction for which no separate genetic control has been identified) to kynurenine, which was before its identification known as the *v^+substance*. This in its turn is converted, under the influence of the cn^+ gene into a '*cn^+ substance*', which is probably 3-hydroxy kynurenine; and after this two further steps of reaction, controlled by the *normal-cardinal* and *normal-scarlet genes*, intervene before the actual brown pigment is formed (Fig. 15.4).

The reactions leading to the brown pigment must be linked in some way with those leading to the red, since some genes, particularly those of the *white* locus, affect them both. Probably this gene is essential in connection with some common substrate or carrier-protein, which has to link up with the cn^+ substance and with something equivalent in the reaction-series leading to the red pigment. It is interesting to note that the cn^+ substance can only produce its effect during a certain limited period of development, extending from sometime after puparium formation for about forty-eight hours. Presumably before that time the kynurenine is not yet avail-

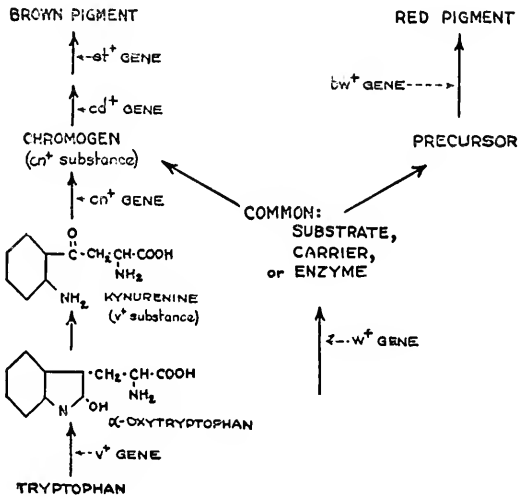


FIGURE 15.4

Eye pigment development in *Drosophila*. (After Beadle 1945.)

able, while by the end of the period of sensitivity it must have been converted into something else. This provides a clear parallel to the restriction of periods of competence found in more usual experimental embryological studies. It is also worth pointing out that phenocopies of eye-colour mutations cannot be easily produced by environmental shocks of a general nature, such as high or low temperatures; the epigenetic system is not complex enough to be unstable with respect to such non-specific agents.

Another well-analysed system of pigmentation is that of the guinea-pig hair colour (Wright 1942). It will not be described here but it well illustrates the point which is being made, namely the real complexity of apparently simple developmental processes.

There is a further important point about developmental paths, or sequences of epigenetic reactions, which has not been adequately illustrated by the examples so far described. That is the existence of sharp distinctions between the comparatively small number of alternative paths which normally occur in the development of a particular species. In embryological studies, we have seen (p. 179) how amphibian gastrula ectoderm is competent to become either epidermis or neural tissue or mesoderm, and in the great majority of cases becomes quite definitely either one or other of them. A similar situation is revealed by some genes in *Drosophila*. For instance, Goldschmidt (1938, 1945) has shown that in flies of certain abnormal genotypes, what would normally be the presumptive wing tissue enters on one of the alternative epigenetic paths, and develops as a haltere; or in another genotype it becomes a leg. If the normal *aristopedia* gene is replaced by its mutant allele (ss^a), the tuft on the antenna known as the arista may develop into the terminal section of a leg, with perfectly developed claws.

Genes which produce this type of effect, in which a part of the embryo develops into an organ which is normally located somewhere else in the body, are known as homeotic genes. A considerable number of them are known in *Drosophila*. Besides *aristopedia*, for instance, *proboscipedia* converts the mouth parts into a leg-like organ; *tetraltera* converts the wings into halteres, while *tetraptera* converts the halteres into wings; *podoptera* causes the wings to become legs, while *bithorax* changes the whole metathorax into a mesothorax. Some of these effects can also be produced by environmental agencies; thus Gloor (1947) found that ether treatment of the young larva a few hours after laying would produce a phenocopy of *bithorax*, presumably by changing the condition of the larval hypodermis at the time when the imaginal buds are first being formed. Similar changes can, however, also be produced at a much later stage since, as we saw (p. 141), abnormalities in the folding of the imaginal buds just before pupation may cause parts of them to develop into tissues which should belong to another part of the body. It is probable then that most of the homeotic genes act in the first place at the time imaginal buds are first formed but that these alterations do not produce any definitive effect until about the time of pupation.

Once a piece of tissue has entered on a path of development, its final condition can be affected by all the genes which are concerned in that path. Thus, if, in an animal in which the arista takes the leg path owing to the presence of the mutant ss^a gene, the genes concerned with the development of the leg have been replaced by mutants such as *fourjointed* which produce shortened legs, then the aristal leg will also be shortened; while

genes which usually affect the arista will now have no effect on it (Waddington 1940c). This shows that, as might be expected, the activity of a gene is not determined simply by the geographical location within the body, but by the type of developmental process which is going on (Fig. 15.5).

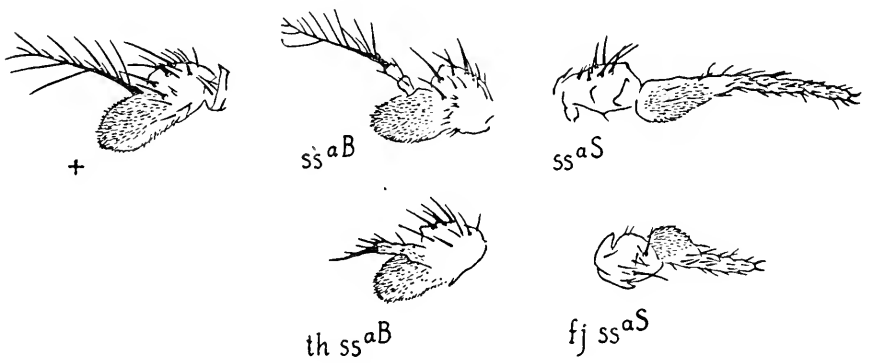


FIGURE 15.5

The mutant allele ss^{aS} in *Drosophila* converts the whole arista into a leg, while the allele ss^{aB} changes only the proximal part into a leg, the distal part remaining an arista. A gene *th* (thread) which normally removes the branches from the arista, affects only the arista-like portion in ss^{aB} flies, and *fj* (four-jointed) which shortens the legs also shortens the leg-like arista of ss^{aS}. (From Waddington 1940a.)

Primary and secondary effects of genes

Some of the studies we have mentioned, particularly that of Beadle and Ephrussi on the eye colour genes, were at one time thought to hold out the hope of leading us to an understanding of the primary, immediate effect of a single gene. This has turned out, so far, to be illusory. It has in no case proved possible to be absolutely sure that the gene-effect we can see is the primary one. The subject is discussed more fully later (p. 379), but it is important at this stage to consider some general points about the kinds of primary and secondary effects which genes may have on the development of organs and tissues.

During the development of a complex animal, any alteration produced by a gene at an early stage may have many later repercussions, perhaps in quite other organs than that in which the original effect occurred. A very obvious case is that in which a gene affects an organ of internal secretion. For instance in the mouse a certain gene impairs the secretion of the growth hormone by the pituitary, and, as might

be expected, this affects all these structures for which the hormone is important, so that the animal develops as a dwarf.

Grüneberg (1948) has shown that many other cases in which a gene has manifold and widespread effects can be explained in a similar way. One of the best-known examples is that of a certain lethal in rats. Animals homozygous for the gene show a very diversified complex of symptoms, which eventually lead to their death at an early age. Grüneberg (1938) argues that all (or nearly all) the symptoms can be plausibly considered to be secondary consequences of an original hypertrophy of the cartilage. The network of causation which he postulates in this case is shown in Fig. 15.6. Falconer, Fraser and King (1951) have described another case, in which a gene '*crinkled*' in mice produces a large number of different effects which they suggest can be almost entirely accounted for as the results of a suppression of hair follicle formation between twelve and a half and seventeen days of gestation and after birth. The suggestion that all the effects in this case are actually secondary consequences of one initial abnormality receives very strong support from the fact that another different gene *tabby* also produces (in single dose) exactly the same syndrome. If each symptom was brought about by a separate reaction of the gene, such a parallelism could not be expected unless the *crinkled* and *tabby* genes were identical, which the genetical evidence shows them not to be; whereas if there is one single underlying cause for the syndrome, it is easy to imagine that two or more different genes may affect the basic process and thus produce the same complex end-result.

Hadorn (1948*a*, 1950, 1951*b*) has paid particular attention to the 'pattern of manifestation' of a gene, i.e. the particular collection of organs whose development it alters. He studied certain 'lethals' in *Drosophila*, that is genes whose effects are so profound that individuals homozygous for them do not survive. He emphasises, first, that there are critical periods of development at which death tends to occur. Thus many lethals are known which produce death at the end of the embryonic period, and again there are many for which the time of death is at puparium formation, or at emergence; but there are very few which kill during the middle of larval life. These sensitive periods are the times when a great deal is going on in the epigenetic system, so that slight abnormalities in the tissues, which may have been produced considerably earlier, will then cause drastic effects. They have also been referred to as 'epigenetic crises', and are of various grades of severity.

Hadorn went on to show that if the phenotypes produced by the lethal genes are closely examined, each gene will be found to cause a characteristic pattern of damage, exhibited either by the death and

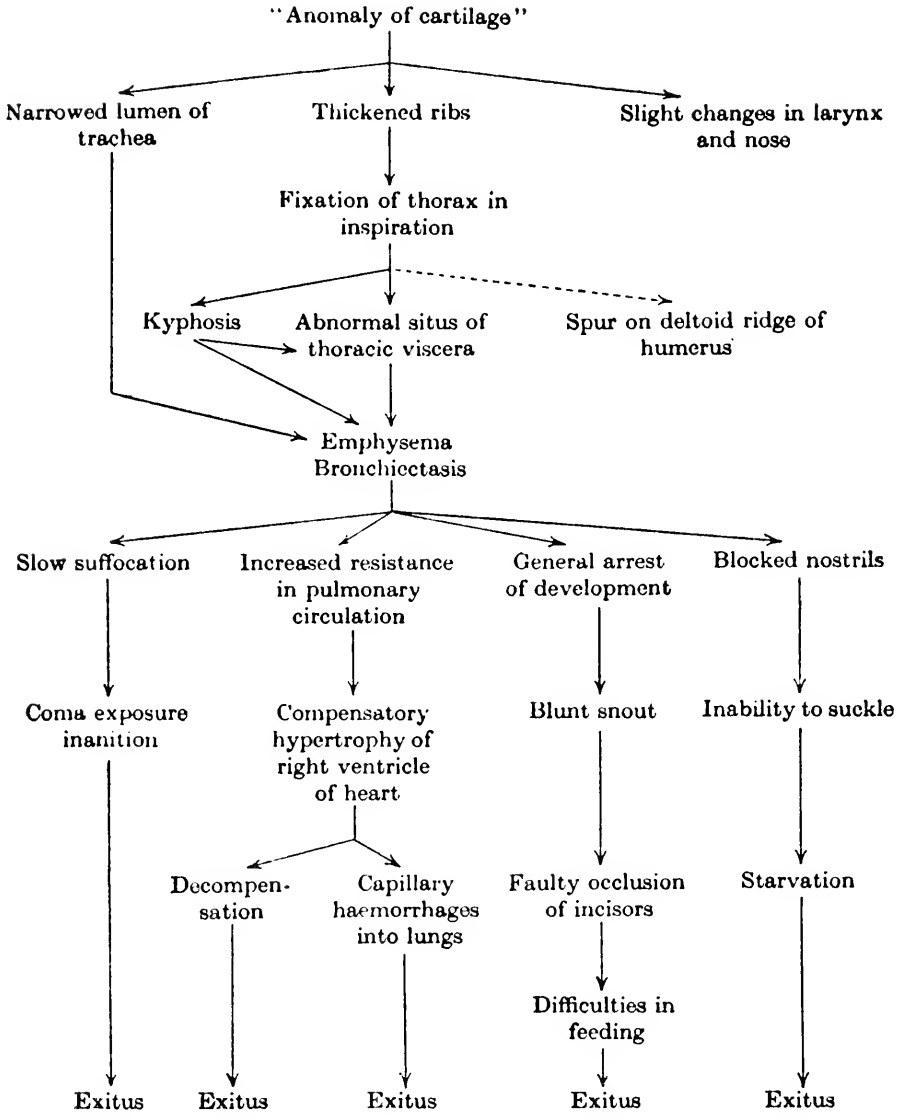


FIGURE 15.6

A gene in the rat causes abnormalities in the formation of cartilage. This has many secondary consequences ('pleiotropic effects'). (After Gruneberg 1938.)

degeneration of the cells of particular tissues, or, when the effect is weaker, by a retardation of growth. This pattern differs in detail in different stocks of the lethal, depending on the other genes associated with it (the 'genetic background'). More important from the present point of view is the fact that the pattern in which the gene normally manifests itself is a mixture of primary and secondary effects. That is to say, in some tissues the abnormality is a direct consequence of the activity of the lethal gene in those particular cells (a primary effect), while in other tissues the gene may either be inactive, or ineffective because its action fails to surpass some threshold, yet the tissue may develop abnormally because it is influenced by unusual substances produced elsewhere in the body (a secondary effect). Hadorn demonstrated the reality of such secondary effects by showing that certain organs from larvae of a lethal type will continue to develop more or less normally if transplanted into host larvae of a wild-type strain. For instance, *lethal-meander* and *lethal-translucida* both die usually at about the time of puparium formation; but the imaginal buds, if transplanted into normal hosts, can carry through a complete metamorphosis. Their death when left undisturbed is therefore a secondary consequence of abnormalities in the development of other parts of the body (probably the protein-metabolising system in the gut of *lethal-meander* and some other nutritive or hormonal peculiarity in *lethal-translucida*).

The distinction between primary and secondary effects can of course also be made within the confines of a single organ. For instance, if the primary effect of a gene is to cause the absence of large parts of the anterior and posterior regions of the wing (e.g. *Beadex* or *Lyra*) a secondary consequence is that when the pupal contraction occurs the longitudinal veins become squeezed nearer together and diverge at a smaller angle (Waddington 1940*b*).) The fact that in Hadorn's cases the secondary effects occur in different organs to the primary ones is not the essential point of the distinction, but merely makes it easier to recognise which effect is nearer the very first influence of the gene on the sequence of developmental processes.

Hadorn has provided a diagram, reproduced in Fig. 15.7, which expresses neatly some of the ways in which secondary effects may occur. In this, the rectangles I, II and III represent three cells in three different organs, as it might be Imaginal Bud, Fat Body and an Endocrine Gland. In each cell ten genes are represented. It is supposed that in each type of cell the heavily ringed genes are active, producing substances (large rings) which fit together into a reaction-chain. Consider what would happen if various of these genes mutated. If the activity of 8 was altered, nothing

would be changed except in organ I, where a primary manifestation would occur. If 3 mutated, that would cause a primary effect in III and a secondary one in I, in the reaction-chain of which an essential link is a product of III, the 'hormone' *b*. If, before the lack of *b* had had irreversible consequences, organ I were transplanted into another host with unmutated 3, then supplies of *b* would be available to it and it would develop normally. Again, mutations in 4, 5 and 6 would cause secondary effects in I and III as

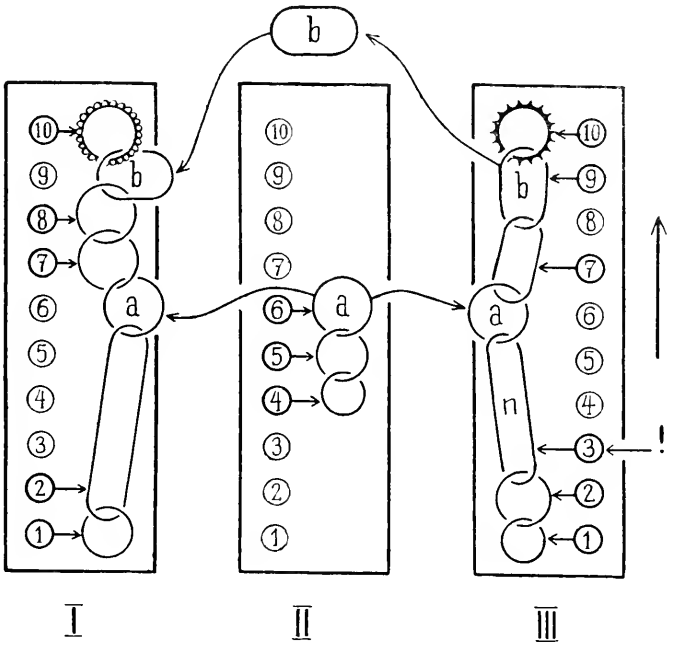


FIGURE 15.7

Diagram of a sequence of gene reactions in three different tissues. See text. (From Hadorn 1950.)

well as a primary one in II. But if a mutation occurred in genes 1 or 2 or 10, both organs I and III would be altered, and these alterations would both be primary ones.

When a gene affects several different organs or tissues it is said to be 'pleiotropic'. The term is not always a very precise one, since in borderline cases there may be difficulty in deciding whether two effects are to be considered the same or different (e.g. in *Beadex* or *Lyra* above). The concept has, however, been widely discussed, since it clearly is of great importance to discover whether any one gene can only exert one type of

immediate effect, or whether a single gene can do different things. It is clear there is no evidence for more than one type of gene-action in cases where the whole of a complex pattern of manifestation can be shown to consist of a set of secondary consequences of some single primary effect (as in the mutation of 3 above, or Grüneberg's rat lethal described on p. 342). Grüneberg speaks of this as 'spurious pleiotropy', contrasting it with 'genuine pleiotropy', in which the same gene would be doing different things in different tissues. The difficulty with this latter category, however, is that at present we have no certain way of detecting it. We can discover cases which seem to be of the type produced by a mutation of gene 1, i.e. in which there are two or more apparently primary effects which are not influenced by other tissues. But there is no way of telling whether the mutated gene 1 is doing the same thing in organ 1 as it is in organ III, and we can at best class this as an 'apparently genuine pleiotropy'.

Most attention, from the embryological side, has recently been given to cases of spurious pleiotropy, since the secondary effects may reveal some of the epigenetic interrelations between tissues.

The primary effects, however, are also not without interest. In a case of 'apparently genuine pleiotropy' we are confronted with two or more at first sight disconnected developmental processes, which are shown actually to be related by the fact that one particular gene is involved in all of them. They must therefore have some fundamental similarities; and the nature of these offers a very important problem which may go to the very heart of the epigenetic systems involved. To take an example. In *Drosophila*, *split* causes both an extra division in the bristle-forming cells (followed by various abnormalities in their arrangement), and also an effect on the facet-forming cells of the eye which is in the main a deficiency in the normal number of cell divisions (accompanied also by some irregularity in arrangement). Now the facet-effect of *split* is almost exactly mimicked by *morula*, another effect of which is to reduce the growth rate of the bristle-producing cells so that the chaetae are smaller than normal. This bristle-effect of *morula*, in turn, is mimicked by *spineless*; and many of the alleles at the *spineless* locus cause as well the 'aristopedia' phenotype, characterised by the conversion of the arista into a leg, the basic alteration being perhaps one on the growth rate and folding of the imaginal bud, We have then a series of developmental reactions, revealed by the mutant phenotypes of *split*, *morula*, and *aristopedia*, which would seem quite disconnected from one another were it not that the overlapping pleiotropies show that there are some basic relationships at the level of apparently primary gene action (Lees and Waddington 1942, Waddington and Pilkington 1943).

Genes may also be used in another way to reveal basic relationships between developmental processes, namely by breeding animals which simultaneously show the effects of the two genes which one wishes to compare. For example, it has been mentioned that in *hairless* and *shaven-naked* some of the trichogens and tormogens are shifted so as to lie side by side, while in *stubble* there is slighter effect in the same sense in all the cell-groups. Now in *hairless-shaven* flies the effect is very strongly exaggerated, and occurs in nearly every group, while in *stubble-hairless* or *stubble-shaven* there is a straightforward summation of the two effects. From this one may conclude that the actions of *hairless* and *shaven* belong to one group and those of *stubble* to another. One cannot be certain of the nature of the relationships within and between groups, but it has been suggested that genes which show exaggeration when combined are those which act at the same time on the same epigenetic process ('homodynamic' genes), while with those which act at different times the buffering of the system reduces the severity of the effects (Waddington 1953).

SUGGESTED READING

Beadle 1945, Ephrussi 1942, Goldschmidt 1938, pp. 3–98, Hadorn 1948*a*, 1950, Grüneberg 1948, Waddington 1940*b*, 1948*a*, Weiss 1947, 1950*b*, Wright 1941 or 1942.

CHAPTER XVI

THE ACTIVATION OF GENES BY THE CYTOPLASM

IT HAS frequently been argued that genes control only the later-developed and more superficial characters of animals and that the development of the basic plan of the body is controlled, not by them, but by the cytoplasm of the egg; and this contention has been hotly disputed by geneticists who seem to feel that it disparages the importance of their subject. We realise now that, as in so many such controversies, both sides are in the right. Undoubtedly within any one lifetime a great deal of the basic pattern of the body is dependent on the configuration of the cytoplasm of the egg; one need only remember such phenomena as the arrangement of ooplasms in mosaic eggs (p. 106), of gradients in the echinoderms (p. 85), or of the formation centre in insects (p. 126). There are indeed many cases in which genetic differences in the nuclei can be shown to have an influence even in early stages of development (e.g. in the merogons or hybrids in frogs, p. 358), but they are certainly not all-important. Thus for embryology the cytoplasm is as fundamental as the genes.

Equally, of course, one must not forget that an egg will not develop into even the general framework of an animal unless it is provided with nuclei. There can be no doubt that differentiation results from the interaction of the division-products of the original zygotic nucleus with the already-present ooplasmic regions of the egg. Neither cytoplasm nor nucleus can be disregarded: in fact the most important subject to discuss is how they affect each other.

We can, then, put into the following form the knowledge with which we have to approach the problem of how development is brought about:

(1) There are local differences with the cytoplasm of the newly fertilised egg. The nuclei, with the genes contained in them, react with the cytoplasm with which they are in contact; and the interacting system of nucleus and cytoplasm may also be affected by substances diffusing from neighbouring regions, as in the process of induction.

(2) As a result of the nucleus-cytoplasm reactions there are formed, firstly new duplicate genes (which allow for the multiplication of nuclei), and also substances of some kind which pass into the cytoplasm and modify it.

(3) These 'immediate gene products' may interact with each other in

the cytoplasm, or with other substances which are already there. There is likely to be quite a complicated series of reactions of this sort before the appearance of the differentiated cytoplasmic constituents which characterise the various fully developed tissues of the adult animal. As these reactions go on, the cytoplasmic environment of the nucleus will be altered, and this will affect the nature of the nucleus-cytoplasmic reactions. The gradually changing constitution of the cytoplasm may also be expected to alter the nature or intensity of the activities of the immediate gene products. We must therefore be prepared to find ourselves faced with a double cyclic system, of the kind pictured in Fig. 16.1.

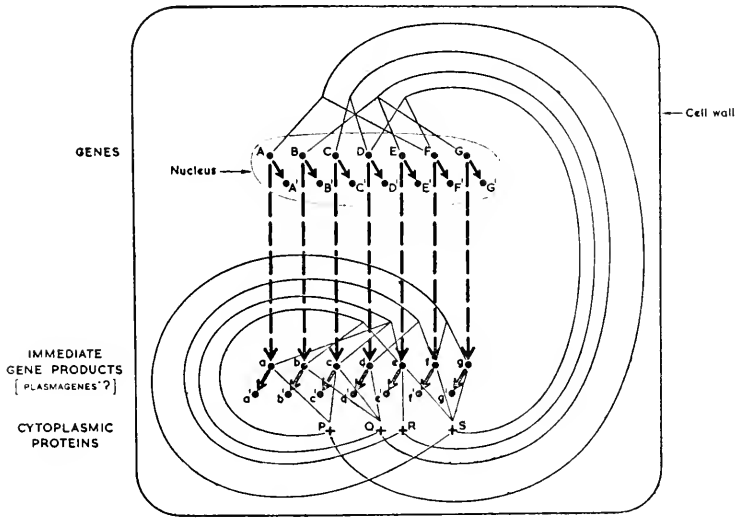


FIGURE 16.1

The double cycle of intra-cellular reactions. The genes in the nucleus acting on cytoplasmic substrates, both reproduce themselves and control the formation of 'immediate gene products', *a*, *b*, *c*, etc. These then use cytoplasmic raw materials (i) perhaps to reproduce themselves identically, in the manner of plasmagenes, and (ii) to elaborate the final cytoplasmic constituents, P, Q, R etc., which are the substrates or raw materials which condition the activity of both the genes and the gene products. (From Waddington 1954*a*.)

(4) The whole system is organised in such a way that development tends to proceed along one or other of a number of alternative canalised paths. That is to say, the developmental reactions tend to go towards one definite type of end-result or another; and while development is going on, the system has some power of self-regulation, so that no effect is

produced by minor abnormalities such as slight losses of material or the substitution of one recessive gene for a dominant one.

It is now necessary to discuss in rather more detail the successive phases in this system of reactions. We shall leave till a later chapter the questions relating to morphogenesis in the strict sense, that is to say, the moulding of the developing tissues into definite shapes. Here we shall be concerned with the processes of chemical change during differentiation. This is one of the most active, important and controversial fields of biology at the present time. There are many different theories which will need to be considered; and since the problems get so far down to the common root of all biological phenomena, light may be thrown on them from very many different angles. We shall have to consider factual material drawn not only from experimental embryology of the old-fashioned kind, but from biochemical studies and from the genetics of micro-organisms as well as higher forms. If is often difficult to assess the relative importance of these various types of evidence. On the one side we may have what seems very precise and definite biochemical information, expressed in terms of nucleic acids, enzymes, phosphate bonds and so on—but we have to ask ourselves just what is the connection between this and the phenomena of development which we are trying to explain, and consider whether it really is more enlightening than theories which operate with less clear-cut concepts (ranging all the way from genes to organisers and ooplasms) which are further from chemistry but nearer to the embryos.

The fourth point mentioned above may be dealt with first. It is a platitude, but an important one, that the body of a multicellular animal is made up of tissues which are rather distinct from one another. Even when changes in the normal course of tissue differentiation are brought about (e.g. by induction, or the action of lithium on the amphibian mesoderm, etc.) the altered tissue is usually switched from one into another of the well recognisable types—from epidermis to neural tissue, or chorda to somite. Intermediate types occur rarely, and when they do (as for instance the 'palisade tissue' found as a 'weak' reaction to a neural-inducing stimulus) they often later develop into something more normal, such as ganglion tissue in the case of palisades. The developmental reactions, therefore, tend to follow one or another of a number of definite paths, which lead to rather well defined and distinct end-states. Further, there is abundant evidence from all the regulatory phenomena which are so common during development, that even if the conditions in a developing tissue are made somewhat abnormal, the epigenetic system is often able to compensate for this, so that the normal end-state is nevertheless

attained. Both these points can be expressed visually by means of a diagram such as that of Fig. 16.2, which represents what has been called the 'epigenetic landscape' (Waddington 1940*a*, q.v. for further discussion).

1. *The effects of cytoplasm on the nucleus*

In earlier chapters we have seen several instances in which localised areas of cytoplasm must have exerted an effect on the nuclei which move

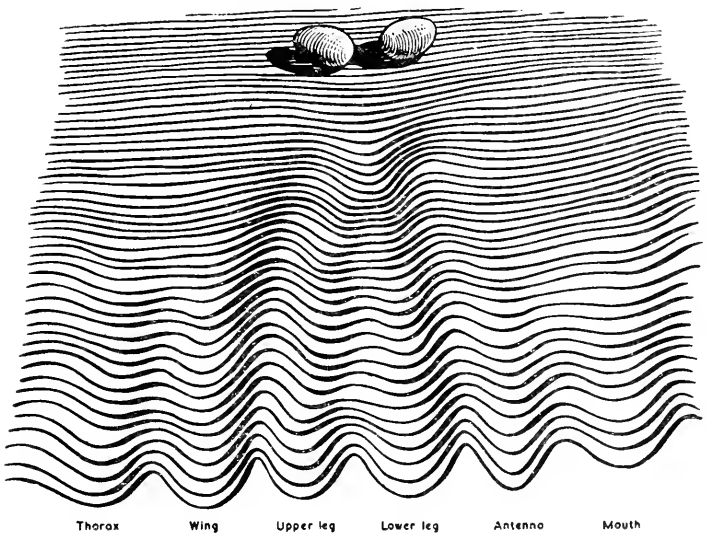


FIGURE 16.2

The 'epigenetic landscape.' A symbolic representation of the developmental potentialities of a genotype in terms of a surface, sloping towards the observer, down which there run balls each of which has a bias corresponding to the particular initial conditions in some part of the newly fertilised egg. The sloping surface is grooved, and the balls will run into one or other of these channels, finishing at a point corresponding to some typical organ. (From Waddington 1954*b*.)

into them. For example, when any cleavage nucleus comes into the formation centre of the *Platycnemis* egg, a reaction takes place and a substance is produced which diffuses forwards to the differentiation centre (p. 126). Again any nucleus which reaches the grey crescent region of the amphibian egg can take part in the development of the organisation centre. And all the mosaic eggs similarly show that the role which a nucleus plays in development depends on the type of cytoplasm in which it lies.

Rather little is known about the nature of the effects which cytoplasm

has on nuclei. There are a few well-known cases in which the reactions produce alterations of the nucleus which are easily visible in conventional microscopical preparations (Review: Mather 1948*a*). One of the best known of these occurs in the eggs of *Ascaris*, a nematode parasite in the gut of the horse. This possesses only one pair of large chromosomes (polyploid races with two or four pairs are also known). These chromosomes are not very typical ones; in place of the normal single centromere or 'spindle fibre attachment' they are provided with a whole series of them extending along the centre section of each long chromosome. A visible nuclear differentiation takes place very early, since from the two-cell stage onwards the chromosomes in most cells break up into small fragments, each provided with only one centromere, and the distal centromere-less ends (which are heterochromatic) are thrown out of the nucleus into the cytoplasm. Only in that lineage of cells which eventually gives rise to the germ cells do the long chromosomes retain their original configuration (Fig. 16.3). Some earlier authors (e.g. Zur Strassen) suggested that the phenomenon was due to factors residing in the chromosomes themselves, supposing that at each cleavage mitosis there was an unequal and orientated division by which a specially coherent chromosome was segregated into this lineage of cells. But Boveri, who was the first to describe this process of 'chromosome diminution', showed by a study of abnormal cleavages in dispermic and centrifuged eggs that the retention of the original structure is dependent on the type of cytoplasm into which the nucleus moves (discussion in Schleip 1929). The type of cytoplasm in which the chromosomes remain coherent can, it is claimed, be recognised not only by its location in the egg but by the presence of fewer vacuoles than there are in the rest of the egg (Bonoure 1939).

Very similar examples of a rapid differentiation of nuclei following a single division can be seen in a few other animals and in the pollen grain formation of some plants.

In certain animals, differences can be seen between the nuclei in different tissues. It is indeed usual for the nuclei to differ somewhat in size, general intensity of staining and perhaps in the number or size of nucleoli, but the significance of these characters is obscure. A more definite type of difference, found in certain groups, is the formation of polyploid nuclei, containing multiples of the normal diploid number of chromosomes. This is rather common in insects (Reviews: White 1954, Geitler 1948). In many tissues the chromosomes divide, and something rather like a process of mitosis may occur in an abbreviated form without any accompanying division of the cell body or of the nucleus. The resulting chromosome threads may lie in a loosely packed mass, or each may adhere closely

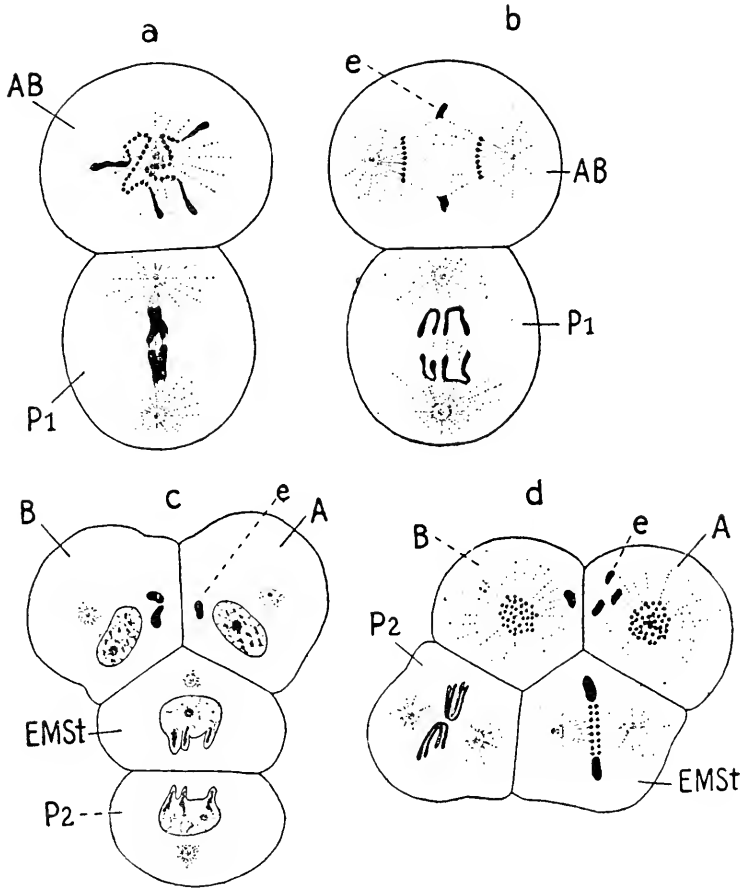


FIGURE 16.3

Cleavage in *Ascaris: a*, beginning of the second cleavage, *b*; later stage of the same cleavage, with the chromosomes becoming fragmented, and their ends (*e*) lost in the cytoplasm, in the anterior *AB* cells; *c*, 4-cell stage from animal pole; *d* later stage of 4-cell stage from the side, when cell *P2* has moved round; chromosome fragmentation and loss of ends is occurring in cell *EMSt*. (After Boveri.)

to its partners. The latter process gives rise to configurations such as those which are best seen in the cells of the salivary glands of dipteran larvae, but which occur in a less perfectly developed form also in the Malpighian tubules, the linings of the gut and elsewhere. In nuclei with such 'polytene' chromosomes, the genes must be represented many times over, and different degrees of polyploidy seem to characterise the different tissues. It is theoretically possible that the relation between the quantity of gene and its effectiveness is not the same for all genes, so that a multiplication

of the whole set of chromosomes would alter the effective balance between the genes. The common occurrence of polyploidy in some form or other in differentiated insect tissues does perhaps suggest that it is related to the varying activity of the nuclei in the different tissues and a similar suggestion has been made, chiefly with respect to plant material, by Huskins (1947) and Huskins and Steinitz (1948). However it must be admitted that most animals and plants which are wholly polyploid, and start life with an abnormal number of chromosome sets in the fertilised egg, do succeed in developing very normally and show little sign that the effective balance of their genes has been altered. Moreover, Staiger and Gloor (1952) have described a lethal factor (*lpl*) in *Drosophila hydei* which has a colchicine-like effect on the mitoses in the cells of the larva, and thus leads to the formation of highly polyploid cells (up to 28-ploid). A similar effect can be produced by cold shock treatment (Gloor 1951). The damaged cells in most cases eventually die, but there is evidence that brain cells, for instance, may retain their differentiation and function as normal constituents of the brain even when their chromosome number is considerably larger than usual; and there is no indication that as the chromosome number becomes altered they tend to assume some other histological type. This makes it difficult to believe that the differentiation of tissues in insects is directly related to ploidy.

A remarkable example of nuclear differentiation has been described by Lindahl (1953) in the echinoderms, in which the micromeres at the vegetative end of the egg become haploid;[1] in this case there is no evidence to what extent this is a cause or a consequence of the differentiation of that region of the embryo. Green (1953) finds similarly that the mesenchyme of the tail tip in anuran tadpoles is haploid.

A very peculiar situation appears to occur in mammals, in which there is evidence that the chromosome number may vary from cell to cell in the same tissue. In insects such as *Drosophila*, on the other hand, the loss of one of the chromosomes from a cell always has a definite effect on its development. Perhaps the apparent ineffectiveness of abnormal chromosome numbers in mammals depends on an ability of substances to diffuse from cell to cell, so that the gene-balance within a large mass of tissue is more important than that within individual cells; or perhaps the abnormalities arise too late in development to have much influence. The question requires much further study (see Beatty 1954).

There is also some evidence of a more biochemical nature which indicates a differentiation between the nuclei in various tissues (cf. Brachet 1952*a*, *b*). Thus in Amphibia, Brachet has shown that the nitroprusside

[1] This has been denied by Makino and Alfort, 1954, *Exper. 10*, 489.

reaction (indication of –SH groups) is positive in all the nuclei of the morula, but almost disappears from the ectodermal nuclei of the neurula, although it still remains strong in the neural tissue and in the notochord and mesoderm. Differences in the nuclei of the various regions of the amphibian gastrula in the incorporation of amino-acids are described on p. 204. Dounce (1954) has investigated the enzymes contained in nuclei isolated from various adult tissues, and although the techniques of isolation and handling of nuclei are not at present absolutely satisfactory, he finds strong evidence that the enzymatic properties differ considerably between nuclei. On the whole the nuclei in a tissue are rich in just those enzymes which are also found in the cytoplasm of the cells and it appears that this cannot be due solely to the contamination of the preparations of nuclei. Again Marshak (1951) claims that the ribose nucleic acid (but not the desoxyribose nucleic acid) of nuclei is very different in chemical constitution in the various tissues.

2. *Effects on chromosomes and genes*

The formation in insect tissues of polytene nuclei, containing such large chromosomes as we see in the salivary glands, makes it possible to inquire whether the fine-grain patterns of the chromosomes are similar in different tissues. A well-formed polytene chromosome consists of a large number of threads lying side by side, each thread consisting of an alternation of refractive and deeply staining segments with less deeply staining stretches; the former contain much desoxyribose nucleic acid, the latter much less. By cytogenetic methods, the position of a considerable number of individual genes has been determined very closely; in fact it may be possible to show that a gene must be located within a certain particular deeply staining segment or band. If the activity of the genes is different in different tissues, it might be that the appearance of the bands would show some signs of this. The investigation of this point is complicated by the fact that the classical type of 'salivary gland chromosome' is the end-product of a long course of differentiation, and in the other tissues of *Drosophila* the process does not usually go so far but stops at an early stage in the sequence. Kosswig and Shengun (1947) were deceived by this into the conclusion that the detailed structure of the chromosomes is very different in salivary glands, Malpighian tubules, gut, etc. Slizynski (1950) showed, however, that all the major landmarks of the chromosomes can be recognised in all tissues in which there is a reasonably good development of polytene chromosomes, so that any differentiation of the chromosome banding must be on a rather minute scale.

Several workers, such as Beerman and Mechelke in Bauer's laboratory

in Germany and Pavan in Brazil, have recently discovered species in which the polytene chromosomes are well enough developed in a variety of tissues for their structure to be studied in detail. They have found clear evidence that individual bands, which may correspond to single genes, may be differently developed according to the tissue in which they lie; moreover some bands can be shown to pass through characteristic cycles of change, which appear to indicate the occurrence of important metabolic events at particular times in development. This is critically important evidence for the supposition, which has always seemed reasonable on general grounds, that the degree of activity of a gene depends on the cytoplasm surrounding it, and varies not only from tissue to tissue, but with the epigenetic situation within any one tissue.

Beerman (1952) has recently made a detailed study of the polytene chromosomes of several tissues of the species *Chironomus tentans* in which they are very well developed. He finds that there are characteristic differences in the general appearance of the chromosomes in the tissues studied; in the salivary gland they are rather compact cylinders, in the Malpighian tubules and rectum they are peculiarly kinked, and in the midgut they have the form of spirally wound flat strips. In all tissues, however, the same sequence of bands can be recognised, so that in all cases the complement of genes appears to be complete, as far as the cytological evidence goes. It is very important to observe that individual bands show characteristic appearances in the different tissues. This can be seen in Fig. 16.4,

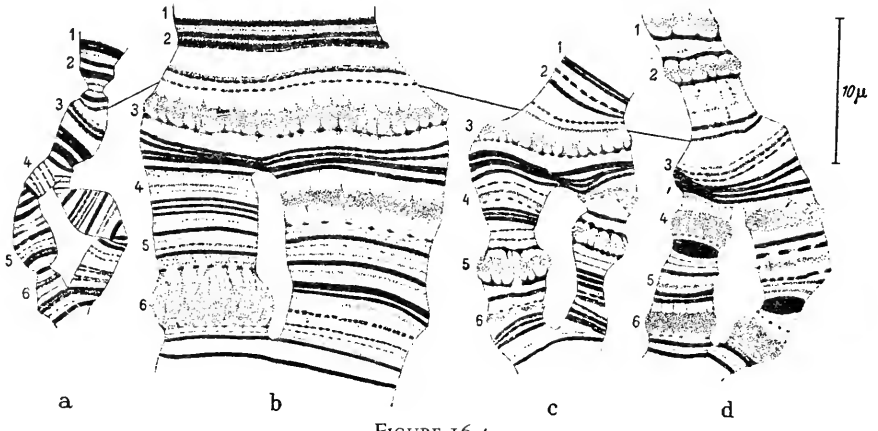


FIGURE 16.4

A certain region in the third chromosome of *Chironomus tentans*, identifiable by a small inversion which causes a failure of pairing: *a* is from a cell in the mid-gut; *b*, salivary gland; *c*, Malpighian tubule; *d*, rectum. Note that the bands (or group of bands) 1–6 are identifiable in every case, but may be differently developed in different tissues. (After Beerman 1952.)

which shows a section of chromosome which can be identified with certainty by the presence of a small inverted section in which no pairing occurs. It can be seen, for example, that bands 1 and 2 appear swollen and 'puffy' in the rectum, but compact in the other tissues, while band 3 is puffy in the salivary glands and Malpighians, band 4 again in the rectum, and so on. Comparable differences may be observed when one compares the chromosomes of the same tissue at different stages of development, for instance at the larval and pupal periods. This is very convincing evidence that the state of activity of a band varies according to the tissue. Beerman suggests that the swollen and puffy appearance indicates a high metabolic activity, and he showed in fact that if larvae are brought out of the cold, in which their metabolism has been reduced, into a higher temperature, there is a rapid formation of droplets, visible by phase-contrast, in the neighbourhood of the most highly developed 'puffs'.

Mechelke's work (1953) was done on another chironomid, *Acricotopus lucidus* in which the salivary gland is subdivided into three lobes, a fore-lobe, mainlobe and sidelobe. All of these contain well-developed polytene chromosomes. In general the banding is rather similar in all three lobes, but there are one or two very clear-cut cases of differential activity, one of which is illustrated in Fig. 16.5. In the mid-larval stages, region no. 33 of Chromosome I in the forelobe is enormously swollen into a fan-shaped

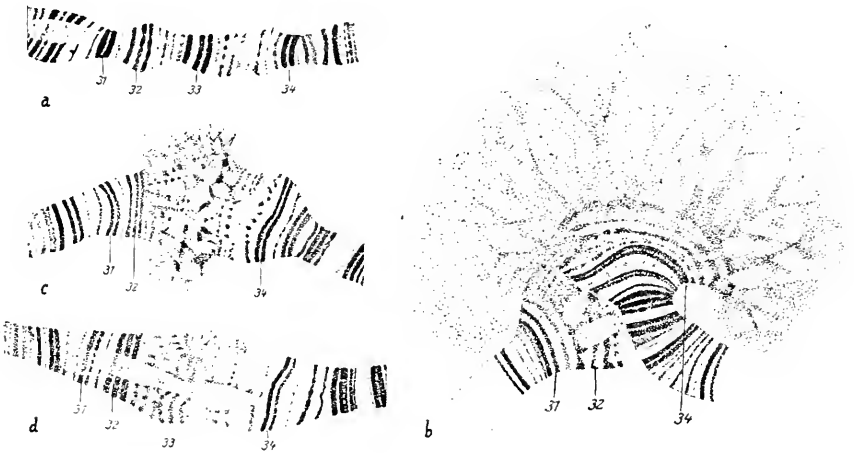


FIGURE 16.5

Figure *a*, the normal structure of part of Chromosome I of *Acricotopus lucidus* from the main lobe of the salivary gland; *b*, the 'Balbiani ring' developed from region 33 in the forelobe of the larva; *c*, a regressing Balbiani ring from the forelobe of a prepupa; *d*, a fully regressed Balbiani ring from a later stage. (From Mechelke 1953.)

mass (a so-called 'Balbiani ring'); in the other two lobes the same region has a perfectly normal structure. The swelling in the chromosomes of the forelobe begins to retract at the end of larval life and during the prepupal stage, exactly at the time when this lobe produces a brownish secretion. In Pavan's case (Pavan 1955) there is also a transitory swelling of particular bands at particular times in certain tissues but not in others. This seems as direct evidence as one could hope for of the activity of individual genes at characteristic times and places.

The fact that a given gene produces a different intensity of effect in different types of cytoplasm is, of course, obvious enough from the mere occurrence of the differentiation of gene-controlled processes. It is also demonstrated very clearly in certain particular cases. Baltzer (1940, 1952*b*, *c*) and his students have carried out many experiments in which an egg of one species of urodele has been fertilised by sperm of some other species e.g. *Triton taeniatus* or *palmatus* egg fertilised by *T. cristatus* sperm. These hybrids are often viable, but some combinations eventually die, usually at fairly late stages of development. A more interesting situation is produced if, after the fertilisation but before the conjugation of the nuclei, the egg nucleus is sucked out with a pipette. This leaves only the foreign sperm nucleus in the cytoplasm of the other species; such animals are known as hybrid merogons. In all the combinations tested, they die before completing development and at an earlier stage than do the corresponding hybrids in which the female nucleus has been left *in situ*. Presumably the origin of the nucleus and cytoplasm in the hybrid merogons from different species makes it impossible for them to interact in a satisfactory manner. In some combinations, e.g. the *Triton* ♀ + Salamander ♂ hybrid (with female nucleus intact), the lethality affects all cells of the embryo more or less equally. In others, however, some tissues may be much more strongly affected than others. In the hybrid merogon in which there is only a *T. cristatus* nucleus in *taeniatus* cytoplasm, it is the head mesoderm which suffers most severely and which dies earliest. This must mean that there is some reaction between the nucleus and cytoplasm of this particular tissue which cannot be properly performed when they belong to different species (Fig. 16.6).

It is noteworthy that in several such cases the merogonic tissue which would die or fail to develop if left *in situ* can be kept alive for a considerable time, and will often continue its differentiation, if it is grafted into a normal host embryo (e.g. Hadorn 1937). Apparently the substances which the merogonic tissues cannot make are diffusible and can reach it from healthy tissue in the neighbourhood. Their nature is quite unknown, and would seem likely to repay investigation.

A very remarkable example of cytoplasmic activation of genes, and one very hopeful for future investigation, occurs in the protozoan *Paramecium*. In this animal, there may be found one or other of a series of antigens, which can stimulate the formation of corresponding specific antibodies when injected into rabbits. *Paramecium* has one great advantage as an experimental animal in that it is possible to arrange for a nucleus of one genetic constitution to get into cytoplasm which has been formed under the control of a nucleus of a different type or under the influence of differ-

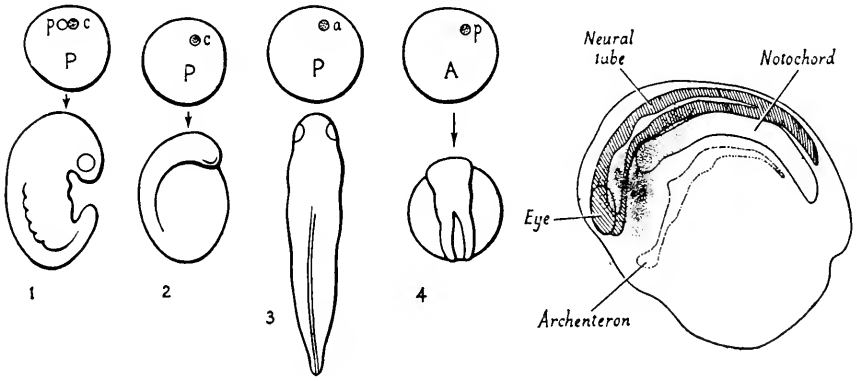


FIGURE 16.6

1. An egg of *Triturus palmatus* when fertilised by sperm of *T. cristatus* develops normally.
2. If the *palmatus* egg nucleus is removed before fertilisation, we obtain a 'hybrid merogon', with a haploid *cristatus* nucleus in *palmatus* cytoplasm; this dies at the early neurula stage.
3. The hybrid merogon combination of *palmatus* cytoplasm with *alpestris* nucleus dies rather later, while (*4*) the reciprocal combination of *palmatus* nucleus in *alpestris* cytoplasm dies earlier. On the right is a diagrammatic longitudinal section through a hybrid merogon of *cristatus* nucleus in *taeniatus* cytoplasm (which behaves like the *cristatus* in *palmatus* combination shown in Figure 2). The dots show the region in which the mesoderm cells become necrotic. (From Baltzer 1940.)

ent environments. This may occur during the process of conjugation, in which two *Paramecia*, possibly of different genetic constitution, come together and each inseminate each other. Very little if any cytoplasm is normally exchanged during this process (though it is possible to arrange for this to happen if the experiment requires it), which therefore gives rise to two individuals, each of which contains a similar hybrid nucleus, but each with its original characteristic cytoplasm.

Experiments of this kind (Sonneborn and Beale, 1949, Beale 1952, 1954) have shown that the immediate control of antigen formation is due

to some factor in the cytoplasm. For instance, when conjugation occurs between two *Paramecia*, one carrying the antigen *A* and the other *B*, the two individuals which separate again will be alike in their complements of genes, but unless some interchange of cytoplasm has occurred, each continues to produce its characteristic type of antigen. However, the responsibility of the cytoplasm for the antigens is not absolute; the genes determine the range of possible antigens which the *Paramecium* can form. So far three main gene-loci have been studied, known as *D*, *G* and *S*. Each strain possesses its characteristic alleles at each of these loci. Normally, only one locus is active at a time. The important point for our present discussion is that it is the condition of the cytoplasm which determines which of the loci shall be in operation (Fig. 16.7). Exactly what is implied

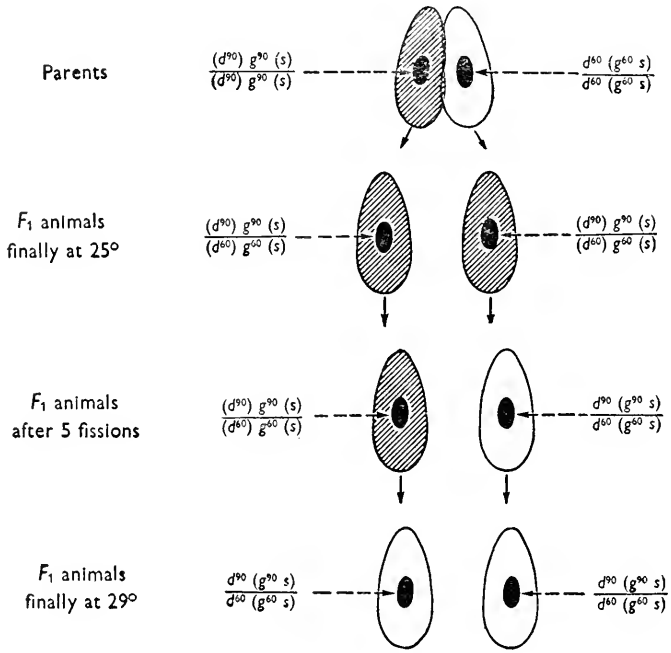


FIGURE 16.7

The results of a cross between *Paramecia* of stocks 90 and 60. The former have been kept at 25° C., and the state of their cytoplasm activates their genes controlling *G* antigens (i.e. g^{90}); while the latter have been at 29° C. and *D* antigens are being produced. In the first few generations of the F_1, the *G* or *D* states of the cytoplasm persist, and bring into action the corresponding *g* or *d* genes in the hybrid: thus the individual with shaded cytoplasm now forms g^{90} and g^{60} antigens, and that with unshaded cytoplasm d^{90} and d^{60}. After some time, the cytoplasm of later generations adjusts itself to the temperature at which the stocks are kept; and when it does so, activates the corresponding genes. (From Beale 1954.)

by 'the condition of the cytoplasm' is still obscure; but, whatever it is, it can be altered by a number of agents, for instance by the temperature at which the strain is being cultured, the amount of food available, the osmotic pressure of the medium, etc. This would seem to open the possibility of discovering how the activation is brought about and the nature of the cytoplasmic properties on which it depends. This is a clear-cut example of the activation, by different types of cytoplasm, of different specifically corresponding genes; and the fact that this occurs, not in different parts of a single body but in the various members of a strain of unicellular organisms does not make the phenomenon any the less relevant to the normal processes of development.

3. *Complete or partial inactivation of genes?*

There is therefore a fairly solid, and increasing, body of evidence which indicates that the nuclei of differentiated tissues become influenced by the cytoplasm with which they are associated. It remains to discuss how far this influence extends. Are we to imagine that certain genes become completely inactivated or even lost; or is it more likely that we are dealing with a merely quantitative speeding up or slowing down of the gene-actions?

There is rather little direct evidence on the matter from the side of embryology. It is true that in many plants almost any part of the organism can be caused to produce a whole plant and must therefore contain the whole set of genes. But plant tissues are not so highly differentiated as those of animals, and one can hardly adduce the evidence of their powers of regeneration to prove that animal cells also always contain a full set of genes. Among animals, it is difficult to find clear-cut cases in which cells can be conclusively proved to have first differentiated in one way and later to have shown a capacity to change into some other type which might be supposed to demand the activity of previously unused genes. When differentiated vertebrate cells are grown in tissue culture (Review: Willmer 1954), they 'modulate' into less-specialised forms which may appear to be dedifferentiated, but they do not re-acquire the ability to develop into some tissue other than the one from which they were originally derived. However, there are fairly convincing cases of such a 'metaplasia' (i.e. a renewal of developmental plasticity) in the ascidians, the embryos of which are highly 'mosaic' at a rather early stage while the adult cells exhibit considerable flexibility during the processes of regeneration and budding (Harrison 1933). We have also seen (Chapter XIV) that there is good evidence for some degree of metaplasia during vertebrate regeneration.

There is a considerable body of evidence from genetics which, although it by no means settles the question, tends to suggest that all genes normally remain in being in all types of differentiated cells.

It is, of course, common to find that a given mutant gene produces a rather localised abnormality and appears to be inactive elsewhere, and if this evidence were taken at its face value, there would be nothing to prevent our supposing that the gene had been completely inactivated or lost in those regions in which it has no visible effect. However, Waddington (1953) has shown that several genes in *Drosophila* are actually in operation in regions in which their influence is not obvious at first sight. For example the well-known gene *vestigial* causes a severe reduction in the size of the wings, but seems to have no influence on the immediately neighbouring thorax. But in flies which are homozygous both for *vestigial* and a gene such as *dachsous*, which does affect the thorax, it can clearly be seen that *vestigial* is active not only in the wings but also in the body (Fig. 16.8). One must assume that the effect of the *vestigial* gene on the thorax normally falls below some threshold and produces no visibly abnormal result unless the development of the thorax has already been upset, and its canalisation weakened, by the action of some other mutant such as *dachsous*. There are certainly many other cases of such sub-threshold effects, as would be expected if all genes are effective, to a

FIGURE 16.8

(On the left). The gene *dachsous* causes a slight enlargement of the thorax of *Drosophila; vestigial* reduces the wings to vestiges, and *apterous* has a still more severe effect of a similar kind. In the fly shown, which is homozygous for all three genes, it is clear that *vestigial* and/or *apterous* have sub-threshold effects on the thorax which become effective in the combination with *dachsous*. (From Waddington 1953.)

(On the right.) Sections through the spermathecae of various mutants, showing how the shape is affected by genes whose main action is elsewhere. (From Dobzhansky 1927.)

greater or lesser extent, in all tissues, though with different intensities in different parts.

A similar conclusion is suggested by the fact that careful metrical study often reveals an activity of a gene in a tissue which it had previously been thought to leave uninfluenced. Thus Dobzhansky and Holz (1943) induced a number of mutations, affecting the eye colour, bristles or other external characters, in long inbred strains of *Drosophila melanogaster*. Each mutant strain thus differed from the race from which it was derived only by the actual mutated gene. By comparing two corresponding races it could be shown that nearly every mutant produced alterations in an apparently quite unrelated character (the shape of the spermatheca) as well as in the eye colour, etc. by which it had originally been detected. It is difficult to suppose that this was merely fortuitous and it seems much more probable that the evidence can be accepted as indicating that all, or nearly all, genes are active in every tissue (Fig. 16.8).

Another piece of evidence tending in the same direction is given by some work of Demerec (1934, 1936) and depends on the phenomenon of somatic crossing over. If a *Drosophila* is heterozygous for two linked genes, such as *singed* (bristles) and *yellow* (body colour), during development a process of crossing over will, in a few cells, take place at a mitotic division, so that one of the daughter-cells becomes homozygous for *singed* and the other for *yellow*. These cells will each give rise to a small patch of tissue, and if this forms part of the body surface, one will see small twin spots, a yellow-coloured one and one with singed hairs. Demerec bred flies which were heterozygous not only for *yellow* and *singed* but also for one of a number of small deficiencies located in the same chromosome. He found that in most, but not quite all, cases the somatic crossing over now gave rise only to a single spot, either a yellow one or a singed one, the other partner spot being missing. This was interpreted to mean that the daughter-cell which had become homozygous for the deficiency (and therefore lacked entirely the genes involved in it) were not able to survive: the deficiency in fact was operating as a 'cell-lethal'. If this is true, it means that all the genes involved in the cell-lethal deficiencies are not only normally operating in the hypodermis cells, but are active in such an important way that in their absence the cell dies. Since the deficiencies were selected at random, and were not known previously to contain genes active in the hypodermis, this is rather good evidence that all genes are active in these cells, most in fact being essential to life. And presumably the same conclusion must apply to all the tissues in the body, although the relative importance of the various genes could not be expected to be always the same.

There are therefore some grounds for thinking that all genes may be active, and producing effects of some kind, in all the cells of the body, even in those in which, owing to the canalisation of developmental processes, the influences of the mutant alleles do not suffice to produce any divergence from the normal. It must be emphasised that this suggestion remains no more than a hypothesis, and that it is quite possible that in some tissues certain genes become completely and irreversibly inactivated or lost: the point will not be finally decided until it is possible to transplant nuclei from differentiated cells into cytoplasms of an earlier and not-yet-determined stage and to discover whether such nuclei still retain the full range of developmental potentialities. Briggs and King (1952, 1953) were the first to achieve any important success in this. Frogs' eggs were parthenogenetically activated by pricking with a glass needle, and the egg nucleus removed (fortunately it can be located with some certainty in this form). A nucleus with a little associated cytoplasm from a cell of a later embryo was then injected into the enucleated egg. Cleavage followed in a fair number of cases. With nuclei taken from morula, blastula or early gastrula stages, complete development of the host egg into a fully differentiated larva sometimes occurred. This proves that, as might be expected, no irreversible change has occurred to the nucleus during these early stages, before the onset of determination, let alone of cellular differentiation.

More recently, similar results have been reported with nuclei from the determined but not yet differentiated tissues of the late gastrula. Waddington and Pantelouris (1953), working with newts' eggs, found that such nuclei were just as good as earlier ones for enabling cleavage to occur, but in their material the host eggs always stopped at the beginning of gastrulation, whatever the age of the transplanted nucleus; this was probably due to the inadequacy of the technique rather than of the nuclei. King and Briggs (1954), however, have succeeded in transplanting such nuclei in frogs, and have shown that they can control the development of completely normal tadpoles. Thus determination does not involve any irreversible loss of gene function; it still remains uncertain how far this is also true of differentiation.

4. *The mechanism of gene activation and inhibition*

One would, of course, like to understand the mechanism by which the cytoplasm influences the nucleus and stimulates or inhibits the activity of various genes. There are several possibilities which must be envisaged. In the first place, so long as division continues in a cell-lineage, the genes in the nucleus must be synthesising duplicates of themselves so as to provide the increasing number of chromosomes; and at the same time and

probably even after all division has ceased, the genes must manufacture substances which pass into and influence the cytoplasm. We shall discuss the nature of these substances in the next section. The point which is being made here is that the genes are producing substances; and in order to do this they must use some raw materials. It is therefore possible that one of the factors which controls the intensity with which the genes operate is the availability of the relevant raw materials.

One form which the competition for raw materials might take would be that the actual total quantities of certain substances set a limit to the activity of particular genes which specially required them. It is perhaps rather improbable that competition of this kind plays an important part in development. When an egg is cut into fragments the amounts of cytoplasmic raw materials available to the nucleus will of course be reduced. Nevertheless, if the cut is made in the right direction a normal embryo may be produced. It seems more plausible to suggest that it may be not the total amounts of substances but rather their relative concentrations which influence the activities of the genes.

There are, of course, other theoretically possible types of control mechanism; for instance some regions of cytoplasm may contain substances which specifically stimulate or inhibit particular genes, somewhat in the manner of co-enzymes or enzyme inhibitors: or the products which the gene passes into the cytoplasm may themselves tend to increase or diminish further gene activity. Our knowledge is so slight that it is hardly profitable to enumerate any more possibilities. It is worth pointing out, however, that it is perhaps at this level that one should look for the explanation of the fact that in any given species of animal there are only a limited number of rather sharply distinct alternative paths of development. We shall show later that this is an expected consequence of any system in which a number of different chemical processes either compete with another for a limited set of substrates, or interfere by other kinds of mutual stimulation or inhibition. We shall see reason to suppose that the cell contains another set of similarly competing or interfering reactions, namely those which operate between the products which the genes pour out into the cytoplasm. It is not clear to which of these two systems—that involving genes and their raw materials, or that involving gene products and their raw materials—the formation of alternative pathways of development is due, but we shall discuss the matter more thoroughly in connection with the gene-product system, which will be dealt with in the next section.

Our information about the mechanism of the cytoplasmic control of gene activity is, perhaps, fullest in connection with the phenomenon of

embryonic evocation; but even so, it is not enough to carry our theories on to firm ground. When a group of embryonic cells is induced to enter on some particular path of differentiation, say towards the formation of neural tissue, we know that the detailed character of the tissues produced will be determined by the specific nature of the reacting cells. In a few cases we can be certain that the specific character of the competent cells is an expression of the genes they contain; for instance when melanophores are induced in genetically white or black axolotl tissue. And it is most probable that in other cases, in which no analysis by genetic methods has yet been made, most or all of the specific differences involved are to be attributed to genes rather than to cytoplasmic factors. We have then good grounds for supposing that when gastrula ectoderm is evocated to form neural tissue, the set of gene-activities stimulated within it are different from those which would be involved in the development of epidermis. We have already described the basic information about the nature of the evocation process (p. 206), but it needs to be examined again from the point of view of gene activation. However, some of the theories about it also involve the products, as well as the precursors, of gene activity, and this discussion also will be postponed till chapter XIX.

SUGGESTED READING

Baltzer 1952*b*, Beale 1954, pp. 77–123, 148–163, Beerman 1952, King and Briggs 1953, Mather 1948*a*.

CHAPTER XVII

THE SYNTHESIS OF NEW SUBSTANCES

IN CONSIDERING separately the influence of the cytoplasm on the genes, by way of activation and inhibition, which was dealt with in the last chapter, and the influence of the genes on the cytoplasm by the production of substances active in development, we have made a distinction which, however convenient, is to some extent artificial. It will be shown later that in all probability the substances produced by genes at an early stage of development are themselves capable of affecting the levels of gene activity at later stages. The gene-cytoplasm complex is a single system, between the parts of which reciprocal interactions occur. But it is easier to discuss the individual steps in such circular reactions separately at first, and to try to put them together again later.

1. *The parts of the cell*

Information about the production of substances by genes comes partly from genetics, but very largely from cytological, embryological and biochemical studies. It will therefore be advisable to begin this chapter by a summary description of the structure of a typical embryonic cell, mentioning the cell-parts which are most important for the subsequent discussion (Fig. 17.1).

The cell nucleus consists of the chromosomes, nucleoli and nuclear sap, all contained in a nuclear membrane. In the chromosomes one can roughly distinguish two types of material (see White 1954): the euchromatin, which shows the typical staining behaviour from which the chromosomes ('the coloured bodies') derive their name; and the heterochromatin, of which there may be more than one kind, whose staining behaviour diverges in various ways. The staining reactions of the euchromatin are in the main due to their content of desoxyribonucleic acid (often abbreviated to DNA) which can be more or less specifically recognised by the Feulgen reagent and less certainly by many other stains. This substance becomes condensed on to the chromosomes at the time of cell division, but it may spread more diffusely throughout the nucleus in interphase. The different staining behaviour of heterochromatin is the result in part of the fact that the phases of the nucleic acid cycle in it are not synchronised with those of the euchromatin. It is probably also due in part to a greater concentration in the former of the other type of nucleic

acid (ribose nucleic acid, or RNA). The chromosomes also contain protein. Much of this is in the form of protamines and histones, two rather peculiar types of proteins which are characteristic of chromosomes and hardly known elsewhere. There are undoubtedly also other types of proteins in chromosomes, but there is as yet little agreement about their nature (cf. Mirsky 1952).

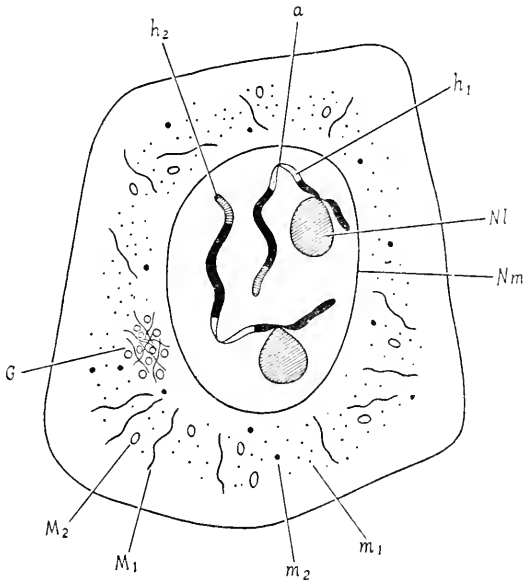


FIGURE 17.1

Diagram of the main elements in cell structure. Inside the nuclear membrane (*Nm*) are pairs of chromosomes; each chromosome may contain not only euchromatin, which stains deeply at mitosis, but also 'heterochromatin', possibly of more than one kind (h_1, h_2); each also possesses a centromere, or spindle attachment (*a*) and some chromosomes form nucleoli (*Nl*) at specific places along their length. In the cytoplasm, there may be a special granular or vesicular region, the 'Golgi apparatus' (*G*). Most of the cytoplasm appears clear, but it contains largish particles, known as mitochondria (M_1, M_2) and probably very small ultra-microscopic particles known as microsomes (m_1, m_2).

Attached to the chromosomes there may be one or more nucleoli. Typically each nucleolus is formed at one definite place on a particular chromosome, a so-called 'nucleolar organiser'. The number of places which are active in this way probably varies in different tissues of the same animal; for instance very many nucleoli may be formed at a number of different places on the chromosomes of the amphibian oocyte nuclei,

while only one or two appear in most somatic cells. The nucleoli contain basic proteins and RNA, but little or no DNA.

The last constituent of the nucleus is the sap. Unfortunately little is known about this, although it must be very important since it presumably contains both the substrates out of which new chromosomes are built and also the immediate products of gene activity. The main constituents are undoubtedly proteins (cf. Brown, Callan and Leaf 1950) and there is no nucleic acid.

The whole nucleus is enclosed by a nuclear membrance, which seems, in several, and perhaps in all, cases to be a double structure. An outer lipo-protein layer, a few hundred Å thick, has a porous structure, the pores having a diameter also of three or four hundred Å; this is supported on a thinner (*c.* 150 Å) layer which shows no obvious structure in the electron microscope and is probably composed of an elastin-like protein (Callan 1951), on the nuclei of amphibian oocytes; Bairati and Lehmann (1952) believes that in *Amoeba* the porous layer lies inside the structureless one). There is some evidence that the nuclear membrane is freely permeable to proteins, which makes it easier to see how the genes can effectively control the functioning of the cytoplasm (Anderson 1953, Stern and Mirsky 1953).

The cytoplasm consists of a clear 'ground substance', in which granules of various kinds are suspended. With microscopes using visible light, it is difficult to obtain much further information about the former. Studies with the electron microscope in recent years have been revealing a variety of laminar or fibrillar structures, often taking the form of very thin double membranes (Fig. 17.2). It is not yet clear, perhaps, to what extent these structures are the results of the types of fixation used to prepare the material (mostly neutralised osmic fixatives, which reveal almost no structure in the nucleus); but it seems certain that there must be quite elaborate structures of some kind or other in the apparently clear cytoplasm. (Sjostrand and Hanzon 1954.)

Most recent discussions have emphasised the importance of the various kinds of particles suspended in the cytoplasm. The first category of these are globules of fat or lipo-protein yolk, which act as reserves of energy-rich raw materials. A more active role is played by the mitochondria, bodies which are large enough to be easily visible in the microscope (*c.* 0·5–3μ in diameter, and sometimes ten or more times as much in length). They contain protein, lipids, and a little RNA. They are usually thought to contain little DNA, but Chayen and Norris (1953) have recently shown that in actively metabolising interphase cells, much of this substance is located in cytoplasmic granules, from which it easily passes

into the nuclei if the cells are damaged, or treated with inappropriate histological methods. The main physiological activity of the mitochondria seems to be the performance of co-ordinated sequences of respiratory enzymatic processes. Electron microscope studies show them to have quite an elaborate internal structure, which again consists largely of closely opposed double membranes (Palade 1952).

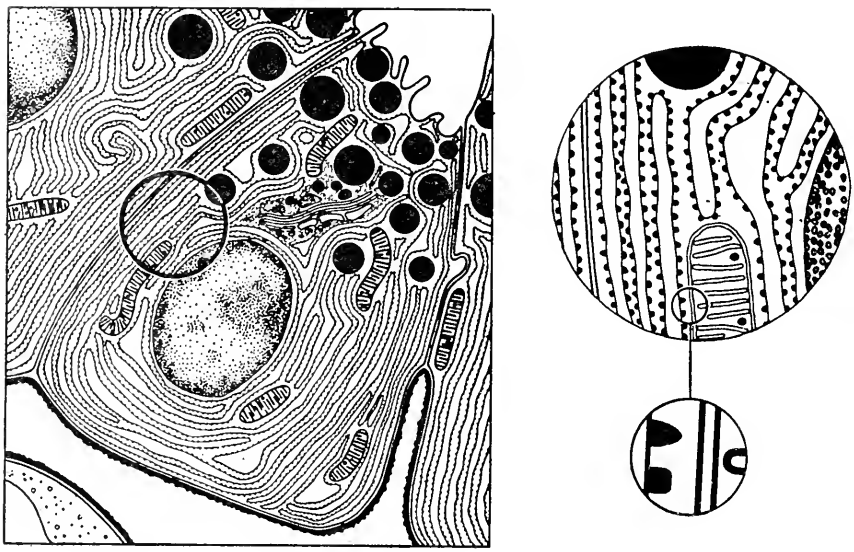


FIGURE 17.2

Diagram of structures seen in the cytoplasm of mouse pancreas cells (electron-microscope studies of ultra-thin sections). The circles to the right show higher magnifications. The sausage-shaped bodies are mitochondria, which have an internal structure consisting of double membranes. (From Sjöstrand and Hanzon 1954.)

The other main class of cytoplasmic granules generally found in cells is referred to by the term 'microsomes'. They are typically at or just below the limit of visibility in the ordinary microscope (diameter usually 60–150 mμ) but can be sedimented out of the clear cytoplasm by ultra-centrifugation. They contain little lipid, and are mainly characterised by their richness in RNA. In addition they contain protein, but usually show little enzymatic activity, except that if the cell from which they are isolated is of a kind in which a specific enzyme is found (as trypsin in the pancreas or amylase in the salivary gland) then this enzyme may be demonstrated in

the microsomes. We shall see that one of the main theories concerning the chemical processes of development attributes great importance to the microsomes in the synthesis of cell-specific proteins. Their condition in the undamaged or uncentrifuged cell is still uncertain, since they range down to a size which is too small to be observed in living material. It is possible that in life they do not exist as separate particles, but that the 'microsomes' found after high-speed centrifugation are really the products of the breakdown of the membranous structures of the clear cytoplasm which are seen in Fig. 17.2.

In embryonic cells there are often many other granules which do not fall quite clearly into the two classes of mitochondria and microsomes (cf. Holtfreter 1946). As has been pointed out (p. 41), several different kinds of granule may be built in to the cytoplasm of the developing oocyte, coming either from the nurse cells or the germinal vesicle or arising *in situ*. It seems probable that during the early stages of development some of these are being gradually transformed into the typical forms of mitochondria and microsomes. Before this transformation is complete, they are referred to either by special names specially invented to cover particular cases (e.g. 'α-granules' and similar phrases) or by general terms such as 'lipochondria', etc. It is possible, also, that the microsomes may gradually develop into mitochondia (Brachet 1952).

2. *Arguing from the gene to the substance*

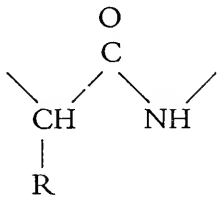
There are two main methods of approach to the problem of the production of substances by genes; one which starts from the genes and attempts to link them up with substances which can be identified as their immediate products, the other which starts from substances which are known to be under genetical control and tries to delve behind their antecedents until it reaches the genes. We shall consider the former approach first; it has so far not proved very productive.

Logically our first step should be to define the gene. Nowadays it is not easy to do this in an unequivocal fashion. Originally, a Mendelian factor was an abstract entity invoked to explain the numerical relations among the offspring of crosses. Then it was discovered that the factors are carried by chromosomes and the term 'gene' was introduced to refer to the physical entity which constitutes a unit factor. The difficulty is to know how to distinguish one unit factor from another. There are several possible criteria. The best known is based on crossing-over. Two genes are considered different if crossing-over can take place between them. One difficulty with this definition is that certain regions of chromosome are known (e.g. the *Y* chromosome in *Drosophilia* or the central parts of

certain chromosomes in *Oenothera*) in which no crossing-over occurs. Further, the definition involves us in proving a negative. Shall we be satisfied if no crossing-over occurs in one thousand individuals, in ten thousand, one million, or how many (cf. pseudo-alleles, p. 375)?

Another criterion could be based on the breakages induced in chromosomes by x-rays or mutagenic chemicals. This would suppose that breaks must occur between genes and not through them and that the breaks would therefore suffice to separate the individual genes from one another. Such a criterion is, however, not easy to use in practice. Thirdly, we might try to base a definition of the gene on its physiological action, a gene being the smallest element which behaves as a unit in the developmental activity of the chromosomes. We shall see, however, that the evidence shows that this definition conflicts with the others, since parts of the chromosome which behave as separate units from the point of view of crossing-over may nevertheless influence one another's developmental activity. It may be possible to interpret these influences as secondary interactions of neighbouring genes, but still the situation makes it difficult to provide a perfectly simple and clear-cut definition.

If, however, one looks more closely at the material structure of the chromosome which one is talking about, the reasons for this difficulty become easier to understand. The chromosome consists largely of protein which, throughout most of the life-cycle of the cell, is combined with a greater or lesser amount of nucleic acid. Now the basic structure of both protein and nucleic acid consists of a linear arrangement of small units. More is known about the proteins. The basic element here is a polypeptide link, the sequence in which is



These are arranged in series which may contain many hundred individual links. Within such series there is a hierarchy of periodicities of different scales. The amino-acids (R) attached to the polypeptide chain may, for instance, be arranged in a repeating pattern, the repeat unit covering a fairly small number of individual links. Then there may be rather larger units corresponding to the unit cells of protein crystals. The protein molecules, as they may exist in solution, are a larger unit again. Virus

particles provide a model of repeat units of a still larger size. The smallest recognisable units in chromosome structure (the bands in salivary gland chromosomes) are still larger. One can represent such a sequence by a series of letters such as *a b c d e′ f′ g′ h′* i′ j′ k′ l′ *m n o* P Q R *S T U* V′ W′ X′. Here the individual letters represent the smallest repeat unit. The groups which are plain, dashed or underlined represent the next largest and lower-case and capital letters represent a still longer periodicity. In a protein structure of this kind there are many types of units, and different ways of defining the gene may lead to different results. We might in some cases find that all the lower-case letters were acting as one unit, while in other circumstances it might be the dashed lower-case letters which behaved separately to the undashed ones. There might even be an overlap between genes, in some cases the lower-case and the capital letters behaving as two units, while in other processes it was the underlined letters which went together. The usual crossover gene is thought to contain some three hundred of the small polypeptide links so there is plenty of room for complications of this kind. The nucleic acid is also a linear structure, and it seems likely that in it too the order in which the constituent groups are repeated can determine structures at least as complex as those of the proteins (Davidson 1954, SEB Symposium 1947). It may well be, indeed, that the factor which operates as a gene is a certain arrangement of chemically reactive places, which may sometimes be incorporated in a length of protein molecule, at other times in a nuclear acid fibre and at other times in the combination of the two. For our present purpose of investigating the nature of the reactions in which genes participate, we may be content to keep in the back of our minds the ambiguities in the precise meaning of the word and to understand it as referring to some sort of small section of chromosome which is acting as a unit.

One line of investigation into the activities of genes has attempted to discover something of their chemical nature by a study of artificial mutation. It was shown by Muller in 1927 that penetrating ionising radiation increases the frequency with which genes mutate; and Auerbach and Robson in 1946 found that certain chemical substances have a similar effect. In spite of the enormous amount of work which has followed the lead of these pioneer investigations little has been discovered which is really pertinent to our present problem. It has become clear that genes can be brought into a condition of instability by various treatments, but the effective physical and chemical agents are of kinds which can be expected to affect a large range of different structures, so that the fact that they are active in stimulating mutations does not make it possible to draw

conclusions about the gene which are precise enough to illuminate the nature of gene-activity (Reviews: Lea 1946, Muller 1947, Catcheside 1948, Auerbach 1952).

The induction of high rates of mutation has, however, provided us with an enormous mass of genetic variations which have thrown light on our problem from other angles. Irradiation by x-rays, for instance, frequently causes chromosomes to break and rejoin in abnormal ways. From the study of such chromosome rearrangements, the fundamentally important point has emerged that the behaviour of a gene may in some cases be influenced by its position in the chromosome. This is the so-called position effect. Several theories have been proposed to account for it (cf. Lewis 1950, Serra 1949). There are two main hypotheses, which seem at first sight to be of rather radically different nature. The first accepts the conventional idea of the gene as a distinct and individual particle and supposes either that neighbouring genes produce substances which can react together (Offerman 1935, Stern *et al.* 1946) or that they interfere with one another by competing for the same substrates (Waddington 1939*a*); if the diffusion of these substances is slow, it becomes important whether the genes are close together or far apart. The second involves a more profound change in previous ideas. It suggests that gene-activity is not to be attributed to circumscribed particles, which could be considered as separate 'beads along the chromosome thread', but that the basic elements are short stretches of chromosome which are not sharply bounded off against each other, but rather shade into or overlap one another. A change in the order of the chromosome thread will in that case alter the character of the fundamental reactions carried out by it (cf. Goldschmidt 1938, 1946).

It is not easy at present to decide finally between these two theories; it is significant, for instance, that Pontecorvo (1950) was led by a variety of the first theory to postulate that genes which take part in a series of reactions in which only a few molecules are involved in each cell will tend to lie close together, like successive machine tools on a production line; a special attempt was made to find such genes in a mould, *Aspergillus*; several genes controlling the production of biotin were duly discovered, all located very close to one another; but by that time Pontecorvo was feeling tempted to interpret the phenomenon by the second theory rather than by the first, which had led him to predict it (Pontecorvo 1952*a*, *b*).

The study of multiple allelomorphs has recently produced evidence which seems to reinforce the theory which postulates less definitely defined genes. It has long been known that there may be many different mutant forms of the same 'gene', if one may use the old terminology for the moment without begging the question. At one time it was thought

probable that the different alleles differed only quantitatively, in efficiency or even perhaps in quantity of substance (cf. Goldschmidt 1938). More recently, several cases have been described in which the differences must be qualitative. For instance, Stern and Schaeffer (1943) showed that there must be two aspects to the activity of the gene *cubitus interruptus* in *Drosophila*, an ability to combine with a substrate, and power of reacting with the combined substrate; and they demonstrated that in the various alleles of this locus these two properties vary independently, some alleles having a high combining ability and low reactivity, others the reverse. Similarly, Waddington and Clayton (1952) found that the various alleles of the gene *aristopedia* in *Drosophila* vary independently in their effectiveness in altering the legs and the antenna of the animal, and cannot be arranged in any single quantitative series.

Recent work has, however, gone much further than such demonstrations that mutation may produce qualitative, and not merely quantitative, changes in genes. In the last few years, an increasing number of cases have been found in which crossing-over takes place between two genetic factors which according to all other evidence would seem to be alleles of a single locus (Review: Lewis 1951). The existence of such 'pseudo-alleles' is beginning to appear so widespread that one is bound to suspect that all apparent alleles may really be of this nature, that is to say, that the quantitatively or qualitatively altered action of each type of mutated gene is correlated with the particular stretch of chromosome which has become changed. If this is so, we shall have to envisage the genetic unit of activity as something which is considerably larger than was previously thought, a point of view which has been strongly urged for some years by Goldschmidt (1938). It remains very difficult to form a picture of the chemical nature of such active units, which would be much larger than normal protein molecules and perhaps similar in size to some of the viruses.

There is another category of position effects which also suggests some rather specific conclusions about the action of genes. It is quite commonly found that when a chromosome is broken and rearranged in a way which brings the heterochromatin into an abnormal position, the functioning of the genes which are now near to it becomes unstable, so that in some cells the genes function with full activity, while in others they are more or less inhibited (cf. Lewis 1950). This gives rise to a variegated or mottled effect, the degree of mottling varying somewhat from tissue to tissue or even in different parts of the same organ. Since many different genes show the same kind of behaviour in such rearrangements, it seems that the heterochromatin must exert some general influence on the activity of most or all genes; the nature of its action remains obscure, but is probably connected

with the importance of RNA in protein synthesis (cf. Serra 1949, Schultz 1952).

The 'inactivation' of the genes in these 'unstable' chromosome rearrangements is probably to be understood as a mutation to an inactive allelomorph, since in certain cases at least it may occur in germinal tissue and then breed true in the inactivated form. It has been suggested by McClintock (1951) that differentiation might depend on the occurrence in the different tissues of gene-mutations controlled by some mechanism of this sort, involving an interaction of heterochromatic and euchromatic segments of the chromosomes. But the hypothesis appears rather far-fetched. In the examples known at present the mutations occur in a disorderly fashion, giving rise to flecks and spots which have little relation to the main anatomical features of the organism. Moreover, to explain differentiation we should need not only the orderly mutation of one gene, but of the whole complex set of genes active in the tissues concerned.

Another mechanism which may be related to gene mutation should be mentioned. Some years ago Avery showed that the characters of certain strains of bacteria (*Pneumococcus*) can be transferred to other strains by cell-free extracts. The strain thus 'transformed' continues to multiply in its new form. The 'transforming principles' have been shown in some cases to be composed of DNA, apparently unmixed with compounds of any other type. A considerable amount of work has been done on the genetical analysis of this most interesting phenomenon (cf. Ephrussi-Taylor 1951) but the mechanism of the transforming action is still quite obscure: the principles may operate by inducing specific gene mutations or in some other way. There are obvious analogies between these bacterial transforming principles and embryonic evocators, but there is little means of deciding as yet whether these are merely formal parallels or whether there is in fact any important similarity between the two mechanisms. The occurrence of embryonic induction by unnatural evocators would at first make it seem unlikely that the two phenomena are closely related; but this argument would have little force if the unnatural evocators operate by setting free the normal evocator from an inactive complex.

3. *Arguing from the substance to the genes*

The alternative mode of approach to the problem of the production of substances by genes is to identify the substances and to try to trace them back to the genes. A very great deal of information has been obtained in this manner, but there is a fundamental difficulty in deriving a complete theory in this way, because we can never be certain, as we trace a substance back through its precursors, that we have reached the last stage from

which we are justified in leaping direct to the gene itself: there is always the possibility, which is indeed often a probability, that there are several intervening steps between the gene and the most deep-lying precursor which we have been able to find.

Most gene-controlled substances which can be easily identified are found in the cytoplasm, and are probably produced in it, so that the genes must be involved only at second hand in their formation. Direct evidence of the production of developmentally active substances by the nucleus itself, or its immediate neighbourhood, is, however, available in some cases. One of the most striking of these occurs in the unicellular alga *Acetabularia* (Fig. 17.3). During most of its life-cycle this organism consists of a rhizoid, which is attached to the ground, from which arises a stalk which terminates in an umbrella-shaped hat. There is only a single nucleus, although the whole alga may attain the size of several centimeters or more. Haemmerling (1934, 1953) showed that if the hat is removed, a new one will regenerate. He then cut off the nucleus-containing rhizome from an alga of one species (*A. mediterranea*) and substituted a similar piece con-

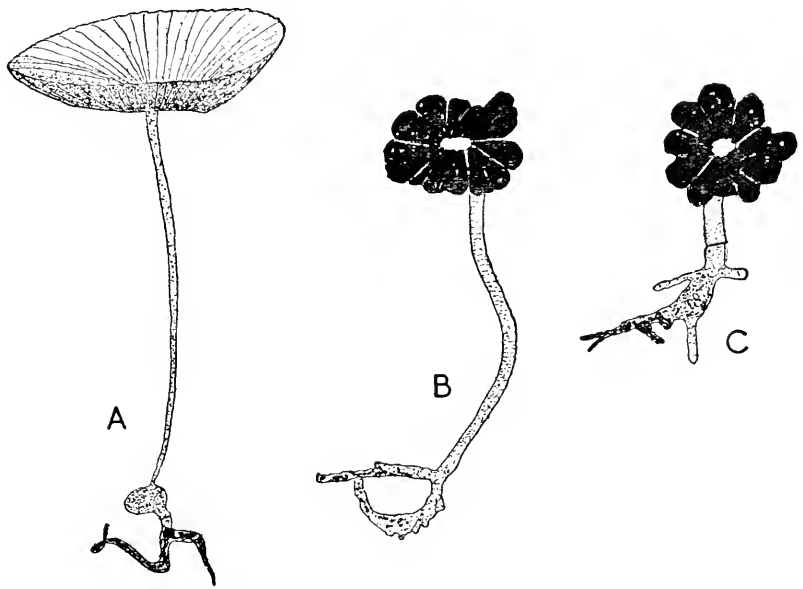


FIGURE 17.3

Nuclear grafts in *Acetabularia*. (*A*) *Acetabularia mediterranea;* the stem has been somewhat shortened in the drawing; the single nucleus lies in the rhizoid at the bottom. (*B*) *A. Wettsteinii*. (*C*) the *Wettsteinii*-like 'hat' regenerated from a short piece of *mediterranea* stem grafted on to the nucleus-containing rhizoid of *Wettsteinii*. (After Haemmerling 1934.)

taining the nucleus of a different species (*A. Wettsteinii*). When the hat was now removed, the important point emerged that the new one which regenerated had the characters of *Wettsteinii*, that is to say of the nucleus lying at the base of the alga and not of the stalk to which the regenerate was attached. It is clear that the nucleus (presumably the genes contained in it) has caused the production of some substance which controls the morphogenesis of the regenerate. Unfortunately nothing is known of the chemical nature of the substances in question.

It has recently been found, e.g. by Mirsky (1951), that different tissues characterised by their richness in particular substances may contain these substances not only in the cytoplasm but also in the nuclei. For instance, haemoglobin is found in the nucleus in the early stages of the development of red blood corpuscles. We have already mentioned such facts as examples of the differential activation of genes by their associated cytoplasm; the point which is being made here is that it also suggests that the substances concerned are manufactured in close proximity to the genes themselves and may be immediate rather than secondary gene products.

4. *Genes and enzymes*

The substances which have been identified in nuclei in this way are mostly enzymes. There is a good deal of other evidence which indicates that genes may frequently operate by means of their effect on cellular enzymes, although as we shall see, these are more probably formed in the cytoplasm rather than directly by the genes. One of the earliest cases in which the effect of a gene could be described in biochemical terms was that of alkaptanuria in man; the homozygote for a certain recessive gene is unable to oxidise homogentisic acid to allantoin, and the former is therefore excreted unchanged in the urine (Garrod 1923). It seems that the normal allele of the alkaptanuric gene is essential for the production of the enzyme which brings about the oxidation. Several other similar cases have been described in mammals, and in recent years very many have been discovered in lower organisms such as moulds, fungi, yeasts, bacteria, etc. (Reviews: Catcheside 1951, Beadle 1949, Horowitz 1950, Haldane 1954). Normal strains of these organisms can grow on relatively simple media, from which they can synthesise all the substances necessary for their continued existence. If a strain, say of the fungus *Neurospora*, is x-rayed or otherwise caused to mutate, and the haploid spores produced are tested for their nutritional requirements, many mutants will be found which cannot grow unless certain specific substances are added to the basic medium. It is concluded that some step in the chain of reactions, which in

the normal strain would lead to the synthesis of the substance concerned, depends on a gene which has mutated and is no longer able to carry out its proper function.

By collecting and testing strains with different nutritional requirements, much may be learnt about the chains of reactions. An example of this is illustrated in Fig. 17.4. The chain of reactions by which arginine is synthesised may be broken by mutations 1 to 7. Mutants 1 to 4 will grow if supplied with any of the substances which occur later (ornithine,

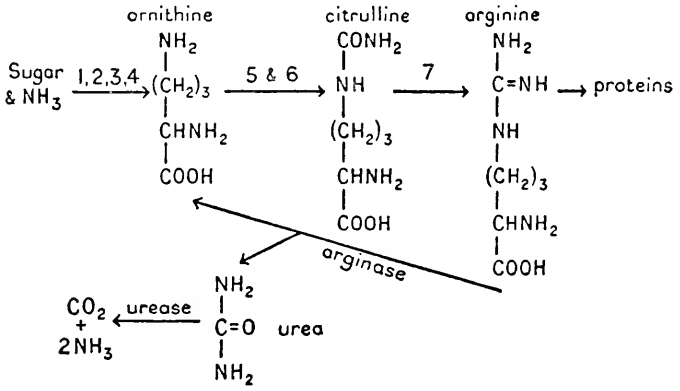


FIGURE 17.4

Metabolic pathways leading to the synthesis of arginine, with indications of the points where different genes in *Neurospora* (1, 2, 3, 4, 5, 6, 7) interfere.

citrulline or arginine); 5 and 6 can grow if provided with citrulline or arginine, but ornithine does not make good their deficiency, while 7 requires arginine and cannot make do with either of the other two substances. Very many similar situations have been discovered.

Since many of the steps in these sequences are known or believed to be carried out by means of enzymes, it is natural to assume that the fundamental activity of the genes involved is the production of these enzymes, and that when the gene mutates the enzyme is either not produced at all or appears in an inactive form. In most cases this suggestion remains a hypothesis; it is rarely that the enzymes have actually been investigated, and when this has been done it has sometimes been found that the relevant one is not absent from a strain in which some reaction-sequence is interrupted, but is present though not effective (Wagner 1949). Nevertheless the postulated gene-enzyme relationship has been generalised and made

into the so-called 'one-gene-one-enzyme' theory. This supposes that all genes operate through the medium of enzymes, and that each gene is connected in the first place with one and only one enzyme. In one form of the theory the gene is supposed actually to produce the whole enzyme; in another the suggestion is only that the gene determines the specificity of the enzyme, and thus its ability to react, and its efficiency in reacting, with the appropriate substrates.

This hypothesis has been widely believed in recent years, and even when some doubts are expressed as to its adequacy, it has usually been given credit for having stimulated a great deal of valuable work. Actually, however, it is probable that the stimulus to the work came rather from the experimental technique of identifying mutations which lead to the blockage of a series of synthetic reactions rather than from the one-gene-one-enzyme hypothesis itself. In fact, almost the first problem which the hypothesis would raise is whether a given gene necessarily controls the formation of the same enzyme in different tissues, in which it is reacting with different cytoplasms. This question, however, still remains quite unanswered and hardly tackled, not, surely, because it is far removed from theory but because it demands a different experimental approach.

The gravest criticism of the one-gene-one-enzyme theory is that it draws its support almost entirely from studies of unicellular or very primitive organisms and thus leaves out of account of the whole range of phenomena involved in regionalisation, which may or may not fall into line with it. Even within the realm of the micro-organisms, it seems that at the present time the theory is beginning to appear as an over-simplification (cf. Haldane 1954). However, work of the kind associated with it has led to the production of numerous genes which have thrown light on a wide variety of problems. For instance, it has been shown that a gene which has mutated to an apparently inactive form may many generations later mutate back again, and the corresponding activity reappears in the cells. This shows that a gene may continue to multiply in a sequence of dividing cells even though it shows no signs of activity; though of course it remains doubtful whether the gene is truly inactive or is continuing all the time to produce an enzyme of altered specificity.

Again, this material provides some relatively clear-cut examples of secondary interactions between gene-controlled processes of the kind which seem necessary as a basis for a general theory of development (cf. Chapter XIX). Thus in *Neurospora* a certain gene blocks the formation of isoleucine and this leads to the accumulation of its precursor, which cannot be utilised but increases in concentration until it inhibits a different but connected reaction by which α-ketoisovaleric acid is changed into

valine (Bonner 1946). Another type of interaction is exhibited by the fact that in certain strains of *Neurospora* and *Aspergillus* which require an external source of *L*-arginine, an addition of *L*-lysine to the medium reduces the effect of the added arginine; and the same competitive inhibition between the two substances is shown by a lysine-requiring strain (cf. Pontecorvo 1950, Emerson 1950).

Finally, we may quote from another field a biochemical example of competition between gene-produced substances (or, less probably, the genes themselves) for a common substrate. One of the earliest attempts to link up gene action with known chemical compounds was the investigation of the genetic control of flower colour (Lawrence and Price 1940, Haldane 1941, 1954). Genes were identified which caused various precisely known chemical changes in the constitution of the coloured substances. Probably these genes act by controlling the formation of enzymes; the evidence is no better, and not much worse, than it is for the similar hypothesis in the *Neurospora* work. Now in crosses involving a number of genes affecting the various different classes of pigments (anthocyanins, flavones and chalkones) it was found that there were interactions in which all the different types of substance were involved. When much anthocyanin was formed, this led to a reduction in the amounts of flavone and/or chalkone. Thus the different gene-activities competed, with efficiencies corresponding to the number and strength of the genes involved, for a limited amount of a common substrate from which the compounds of all those types were derived (Lawrence 1950).

5. *The synthesis of proteins*

Since most, if not all, enzymes are proteins, the evidence from genetics that genes control the formation of enzymes should be linked with such general information as we have about the synthesis of proteins in cells. Although the nature of the chemical processes involved in this synthesis is still almost entirely obscure, there is quite a large amount of rather indirect evidence on the role which various parts of the cell structure play in the production of proteins. Most of this does not come from investigations which have used embryos as the experimental material (for which see ten Cate 1953 and Gustafson 1952) but from studies in the general field of biochemistry and physiology.

It is, in the first place, fairly generally agreed that the nucleic acids participate in an important manner in protein synthesis and indeed are probably essential for it. The synthesis of new chromosomal proteins, which is involved in the reduplication of the genes before cell division

takes place, never occurs in the absence of desoxyribose nucleic acid. In the synthesis of cytoplasmic proteins, it is ribose nucleic acid which is thought to play the active part. Chemical analyses always demonstrate an unusually high concentration of RNA in rapidly growing cells or in those which are actively secreting protein (e.g. some glands, hair-forming cells, etc.). This RNA is largely located in the cytoplasm, but there is often also a considerable enlargement of the nucleoli, which are rich in RNA. For example, nucleoli are absent in the cleavage cells of the amphibian neurula, but appear at about the time of gastrulation, when there is evidence that specific proteins begin to be produced.

There are at present two main theories about the processes of protein synthesis in embryonic or developing cells. The first is that of Caspersson (Reviews: 1947, 1950). It is based chiefly on studies which use spectroscopic methods to take advantage of the fact that the purine and pyramidine bases incorporated in the nucleic acid molecule have very characteristic absorptions in the ultra-violet; this makes it possible for the nucleic acids to be identified within living cells, although it must be pointed out that there are considerable technical difficulties and the method has come in for a good deal of criticism. From his observations, however, Caspersson has come to the following conclusions. The euchromatin, which consists largely of histone and DNA, synthesises replicas of itself and also produces other complex proteins, which could be the agents through which gene-action is exerted: however, the spectroscopic evidence does not suffice to suggest much about them. The heterochromatin (or, more generally, the 'nucleolus-associated chromatin') which contains an important proportion of RNA, is supposed to control the nucleic acid metabolism of the whole cell. It also produces proteins, which tend to be rich in di-amino-acids. These accumulate in the nucleolus, and diffuse from there to the nuclear membrane. On the outside of this, an intensive production of RNA-protein takes place and this is the main source of the cytoplasmic proteins, which are thus supposed to be formed in the immediate neighbourhood of the nucleus (Fig. 17.5).

The other main theory is that of Brachet (cf. 1950, 1952). It differs from that of Caspersson in being derived from a large variety of biochemical, cytological and embryological investigations rather than from a single technical method such as spectroscopy. In its conclusions it lays much more stress on the role of the various types of cytoplasmic particle in protein synthesis. Brachet supposes indeed that the ribose nucleotides formed by the nucleolus (or heterochromatin) do not merely combine with proteins in the neighbourhood of the nuclear membrane, but take part in the formation of microsomes scattered throughout the whole

cytoplasm, and that it is at these particles that the main synthesis of cytoplasmic proteins occurs.

There is as yet, perhaps, no evidence which finally settles the question of whether cytoplasmic synthesis is directly under the control of the nucleus or whether the microsomes are essentially involved. The importance of the nucleus, either at first or second hand, can of course not be

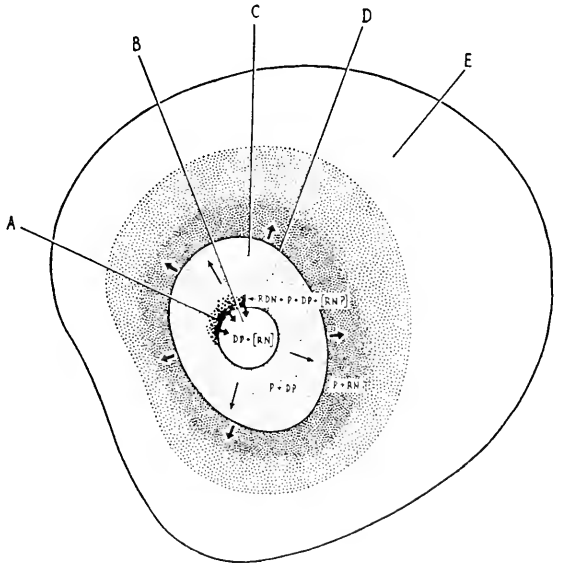


FIGURE 17.5

Protein synthesis according to Caspersson. *A*, the 'nucleolus-associated chromatin', containing ribo-desoxy-nucleotides (RDN), proteins (P), di-amino-rich proteins (DP) and perhaps ribose-nucleotides (RN). *B*, the nucleolus, containing DP and smaller amounts of RN. *C*, the nucleus, in which there is a gradient of P and DP towards the nuclear membrane *D*. In the cytoplasm *E* there is a gradient of P and RN from the nuclear membrane outwards. (After Caspersson 1950.)

denied. For example, Weiss and Hiscoe (1945) have shown that in a neuron growth does not take place throughout the enormously elongated axon, but synthesis occurs only in the cell body in the neighbourhood of the nucleus; the new cytoplasm flows from this region towards the tip of the fibre, and if the axon is constricted, the flow becomes dammed up and a swelling appears proximal to the constriction (Fig. 17.6). The cell body is, however, also the region in which the cytoplasm is most basophilic and probably the site of the main concentration of microsomes, so

that it remains quite possible that the influence of the nucleus is indirect and mediated through them. Again, Brachet and Chantrenne have shown by radioactive tracer studies that if one removes the nucleus from the large unicellular alga *Acetabularia* (cf. p. 377), protein synthesis eventually decreases in the non-nucleated fragment (cf. Brachet 1954). But it is remarkable that the activity continues unaltered for almost a fortnight and remains very considerable for much longer; from this they draw the

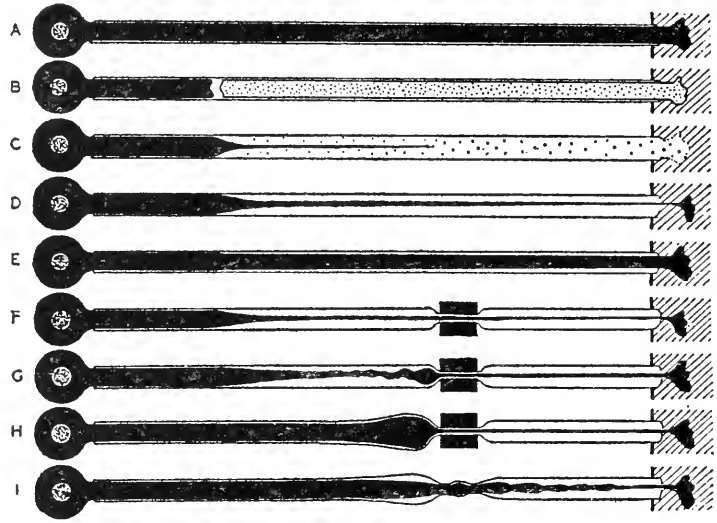


FIGURE 17.6

Diagram of nerve regeneration. Rows *A* to *E* show the normal process when the nerve is simply cut; regeneration takes place from the proximal portion. Rows *F*, *G*, *H* show that if, after the stage of row *D*, the fibre is constricted, the new cytoplasm being synthesised in the proximal (nuclear) end of the cell forms a swelling. When the constriction is removed (*I*) this cytoplasm gradually spreads distally. (After Weiss and Hiscoe 1948.)

conclusion that the nuclear control of protein synthesis is indirect. The final proof that the microsomes take part in the process, and are the actual site of the new production of protein, would be given if they could be transplanted to some unusual location, say to another cell of different developmental fate, and it was found that the protein characteristic of the transplanted material was produced in the new position. Attempts to do this with isolated microsomes have so far been unsuccessful. The results of high-speed centrifugation of ascidian eggs (p. 114) or of echinoderm eggs (p. 90) may, however, probably be interpreted in this way, since

the particles moved under these forces would seem to be comparable with those usually considered as microsomes. The role played by microsomes in the synthetic activity of echinoderm embryos, and their relations with the mitochondria, have been discussed earlier (p. 90).

It will be seen that neither in Caspersson's nor in Brachet's theories is it supposed that the synthesis of proteins occurs actually at the gene inside the nucleus. If enzymes are sometimes produced in this way (p. 355), which is by no means certain, that can hardly be the general rule of protein synthesis. One suggestion has been made (e.g. Wright 1945, Waddington 1939*a*), however, which minimises the difference between synthesis at the gene and synthesis in the cytoplasm. According to this, the genes directly produce substances which are replicas of themselves in most respects (or possibly in all except for the connecting links which hold the genes together in the chromosome); and it is supposed that these replicas pass into the cytoplasm and there control the synthetic processes. This hypothesis has the merit of simplicity, but the evidence for it is slight; the fact that synthetic activity disappears or diminishes when the nucleus is removed suggests that the postulated gene-replicas cannot at all fully take the place of true nuclear genes and it is therefore likely that they cannot actually be replicas in the full sense of the word. It may very well be the case, however, that the immediate products of gene activity have a considerable chemical resemblance to the genes or to some part of them. Possibly the genes produce, as a first step, a product which resembles the protein which, in the chromosome, is combined with DNA, and this, passing into the cytoplasm, becomes associated with RNA, either near the nuclear membrane as Caspersson suggests or in the microsomes of Brachet.

It will be seen from the discussion in this chapter that in spite of the very large effort which has been devoted recently to the attempt to discover how genes operate, and the important body of interesting results which have been gathered, there has really been rather little progress towards the solution of the problem which has been put in the centre of the stage. This may be due to the inherent difficulties of the field; but the biologist who is not primarily a biochemist may be tempted to wonder whether the problem is not perhaps being envisaged in too simple terms. We have tended to think both of genes and of their products as definite and discrete particles, of, perhaps, the dimensions of a protein molecule or a little more. Possibly the active participants in gene-operations are actually of a higher order of complexity than this. As Goldschmidt has urged, and as we have seen above, there is considerable evidence which could be taken to support the idea that the active units in chromosomes

are relatively long structures, rather than 'point-genes'; and electron microscope studies on cytoplasmic structure (Fig. 17.2) suggest that we may have tended also to under-estimate the size and complexity of the active agents in protoplasm. We may, perhaps, find that it is necessary to think of gene action in terms not of enzymes or other protein molecules as we know them in solution, but of extended protein sheets and fibres, which may have properties which largely escape our present biochemical methods. Possibly it is in this way that we shall find an explanation both for the very large number of genes which affect the development of an organ and for the extreme precision with which they control it.

SUGGESTED READING

Brachet 1952*a*, *b*, Haldane 1954, Chapter 2, Goldschmidt 1951, Muller 1947, Pontecorvo 1952*a*, *b*, Spiegelman 1948, Schultz 1952, Wright 1945.

CHAPTER XVIII

PLASMAGENES

IN RECENT years a considerable body of evidence has accumulated for the existence in the cytoplasm of bodies of a more or less gene-like nature. Geneticists have been particularly active in investigating them and have referred to them by a variety of names, such as plasmagenes, cytogenes, blastogenes, proviruses, etc. The first has been the most commonly accepted, and will be used here in a rather wide sense, to cover several rather different types of gene-like entities.

Broadly speaking, plasmagenes are revealed by two different kinds of evidence. On the one hand, breeding experiments may demonstrate that certain characters are inherited through the cytoplasm and not through the nucleus, and thus provide evidence of the existence of cytoplasmic hereditary determinants. Evidence of this kind can be of two grades. We may find that, throughout a number of generations, a certain character follows the transmission of cytoplasm, even when the whole set of chromosomal genes is removed by crossing. For instance, if a female of type *A* is crossed to a *B* male, and her female offspring again backcrossed to *B* males, and so on for several generations, the *A* chromosomes will gradually be replaced by *B* ones, and if any characteristics of *A* still remain in the offspring after several generations, one may conclude that they are dependent on hereditary factors transmitted through the egg cytoplasm and capable of continued multiplication in the absence of their corresponding *A* genes. On the other hand, it may be found that although a character is transmitted only by a parent which contributes cytoplasm to the offspring, and is thus directly dependent on a cytoplasmic determinant, nevertheless this determinant can persist only in an organism provided with the appropriate gene. In such cases (a good example is the 'killer' character in *Paramecium*) we have to do with a gene-dependent plasmagene.

A different type of evidence for the existence of plasmagenes appears when it can be shown that a character can be transmitted from cell to cell by inoculation or other treatment with extracts which do not contain functional chromosomes; we may then conclude that we are confronted with a determinant, presumably derived from the cytoplasm, which can persist and impress some definite character onto the living cells into which it is introduced. The classical examples of such types of behaviour are the

transmissable viruses. When from such evidence we deduce the existence of a plasmagene, it is presumably implied that the cytoplasmic determinant is a fairly complex body, probably of the order of magnitude of a virus particle or a gene. Considerable caution should be exercised in making such deductions. Many years ago, in the early years of the investigation of cancer-producing viruses, it was pointed out that, given a tissue which has an appropriate competence, a particular type of cellular differentiation could be transmitted through an indefinite series of inoculations by means of cell-free extracts whose operative factors, however, were quite simple molecules which acted as evocators (cf. Needham 1936*b*). One knows now that the effective molecules might be even simpler than was realised at that time. It would be quite possible to carry on an indefinite series of transformations of gastrula ectoderm into neural tissue by means of inoculations of cell-free extracts, provided only that these extracts were sufficiently acid. Moreover, one might easily obtain phenomena which simulate a mutation of the virus. If the extracts came to contain free ammonia they would transform the gastrula ectoderm not into neural tissue but into derivatives of the axial mesoderm.

More recently Lederberg (1952) has drawn attention to the same source of possible error. Again, Pollock (1953) has suggested a mechanism for the operation of self-perpetuating and even growing systems which might very easily be confused with plasmagenes (Fig.18.1). In the form he advances it, the idea depends on the phenomenon of enzymatic adaptation (p. 400); but somewhat similar systems might be produced in other ways. Pollock's suggestion is this; suppose that a substance *A*, supplied to a cell, causes it to synthesise an enzyme *a* which converts *A* to *B*; then suppose that *B* induces the formation of enzyme *b*, which converts *B* (perhaps in combination with other substances already in the cell) into *C*; then that similarly *C* is converted into something else, and that finally a product is produced which is converted again into *A*. Such a system will be set going by the addition of an initial quantity of *A* and will then carry on indefinitely. In fact if the system absorbs energy, more *A* may be formed at the end of a cycle than entered it at the beginning, and the system will be able to grow. Such a system clearly has many of the properties attributed to plasmagenes; but it need not be incorporated in a particle. Thus to be justified in using grafting or inoculation experiments to postulate the existence of a plasmagene, one needs evidence not only that the character can be transmitted by cell-free extracts but that the effective factor in the extracts is a particle of the right order of complexity.

Discussions which on the whole favour the importance of plasmagenes in development will be found in Darlington 1944, Darlington and Mather

1949, Spiegelman 1948, Medawar 1947, Ephrussi 1953; a somewhat more reserved attitude is taken by Brachet 1952*b*, Beadle 1949, Waddington 1948*b*, Sonneborn 1951*a*, *b*, and Haldane 1954. General reviews of the phenomena, less concerned with development, are Lederberg 1952, Caspari 1948, *Cold Spring Harbor Symposia* Nos. 11 and 16, 1948 and 1951, and the symposium published as *Unités biologiques douées de continuité génétique* (Paris 1949).

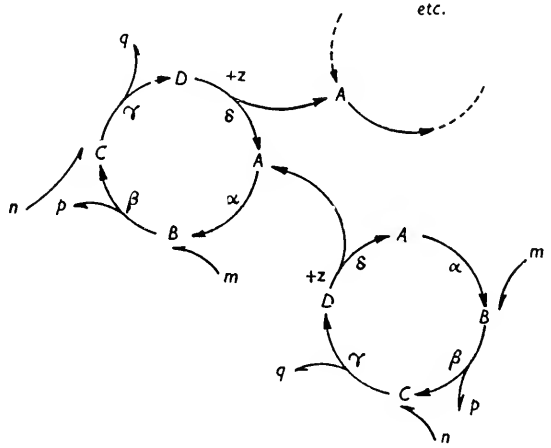


FIGURE 18.1

A self-reproducing cyclic system. It is supposed that when *A* is added to the cell, it induces the formation of enzyme α, which converts it, together with *m* from the rest of the cell, into *B*. After several such steps, during which raw materials (e.g. *n*) are used, and other substances (e.g. *p*, *q*) produced, the system finally absorbs energy (*Z*) and results in the formation of more *A* than was originally supplied. This restarts the original cycle, and also brings into being a new cycle. (After Pollock 1953.)

From the point of view of their possible importance in differentiation, plasmagenes may be considered under the following headings (cf. Fig. 18.2).

1. *Exogenous plasmagenes*

Many viruses, such as those producing disease, are clearly not essential constituents of the animal or plant and are introduced into the cell from outside. There is considerable variation in the ease with which this introduction can take place. Some of the bodies which were orginally thought of as true plasmagenes should be regarded as essentially exogenous factors for which infection is rather difficult. This probably applies to the kappa particles in *Paramecium* (Sonneborn 1947, 1951*a*, *b*). In these Protozoa,

some strains, known as 'killers', produce and secrete into the medium a substance which kills certain other strains, known as 'sensitives'. The production of the killer substance is controlled by cytoplasmic particles known as kappa, and these in turn depend on the nuclear alleles *K* and *k*. The importance of the cytoplasmic particles is demonstrated by the experiment summarised in Fig. 18.3; if *KK* killers are crossed with *kk* sensitives, all the offspring will have the genic constitution *Kk*, but only those which

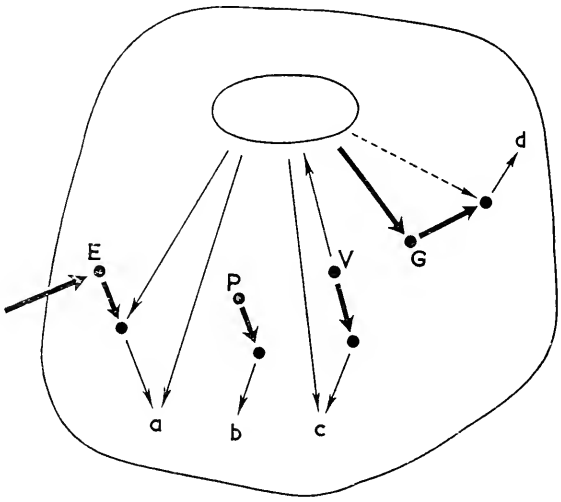


FIGURE 18.2

Types of plasmagene. The diagram shows a cell containing a nucleus, and the following types of plasmagene: *E*, exogenous, originating from outside the cell; can usually multiply only with the co-operation of the nucleus, which may also influence its physiological effect *a*; *P*, a true plasmagene, independent of the nucleus both in multiplication and in effect *b*; *V*, a visible cytoplasmic particle; can usually multiply independently of nucleus, but may affect the latter (cf. *Stentor*), while the nucleus may influence its physiological effect *c*; *G*, a gene-initiated plasmagene, originating under the influence of the nucleus, but multiplying and being physiologically active in relative, though not complete, independence of it.

derive their cytoplasm from the killer parent will contain kappa and behave as killers. If, in such a cross, the period of union of the two Protozoa is unduly prolonged, some kappa-containing cytoplasm may pass into the offspring derived mainly from the sensitive parent, and provided these offspring contain at least one *K* gene, the kappa particles multiply (Fig. 18.4). On the other hand, in cells containing only the recessive *k*, kappa particles which may originally be present fail to multiply and gradually become diluted out of existence as the strain of Protozoa proliferates.

Thus the persistence of kappa is strictly gene-dependent. The particles can be seen in microscopical preparations, following suitable staining. Unlike most normal cytoplasmic particles, they contain DNA, and it seems likely that they should be regarded as invasive exogenous organisms, perhaps comparable to large viruses or rickettsias, rather than as normal parts of the *Paramecium*.

A rather similar case, and this time in a higher organism, is the so-called CO_2 genoid in *Drosophila* (L'Heretier 1951). This is a cytoplasmic factor which causes the individuals carrying it to be highly sensitive to CO_2, which produces in them an irreversible anaesthesia at a concentra-

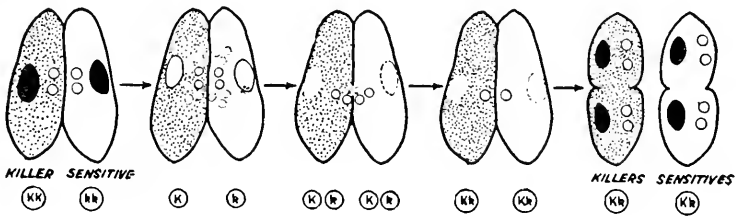


FIGURE 18.3

Cytoplasmic inheritance of kappa.

In the figure on the left, conjugation is occurring between a killer (with genotype *KK*) and a sensitive (with genotype *kk*). In the third figure they are exchanging nuclei and in the fourth and fifth the macronuclei are being reconstructed. Both daughters (now ready to divide) have the genotype *Kk*, but only that with the original kappa cytoplasm will be a killer. (From Beadle 1949, after Sonneborn and others.)

tion which has little effect on normal flies. The agent is easily transmitted by the cytoplasm of the eggs, and can also pass through the sperm, although its passage through the male is very irregular, presumably because of the small quantity of cytoplasm carried by male gametes. There are no genes known with a clear-cut effect on its propagation, but it certainly multiplies more easily in some genetic stocks than others, so that it too may be considered as gene-dependent. There seems no reason to think of the *Drosophila* genoid as anything other than an exogenous virus.

For many other viruses diseases, there is as yet little evidence of nuclear control of susceptibility or resistance. On general grounds, however, it is probable that there is always some variation in this respect and this will probably be under the control of numerous nuclear genes, each of small effect. Again, the physiological result of infection with the exogenous particle in some cases clearly depends on the nuclear constitution of the

individual involved. The classical example is the virus-like particle in the King Edward race of potatoes, which has little effect in that stock but which, when transferred to other races of potato by grafting, produces the symptoms of a severe virus disease (Salaman and Le Pelley 1930). In other cases such variation in effect is less in evidence, but it seems likely that careful search would always reveal some degree of variability of this kind.

2. *True plasmagenes*

One can pass by more or less insensible gradations from cases in which infection is easy and the infecting particle obviously foreign, to the other

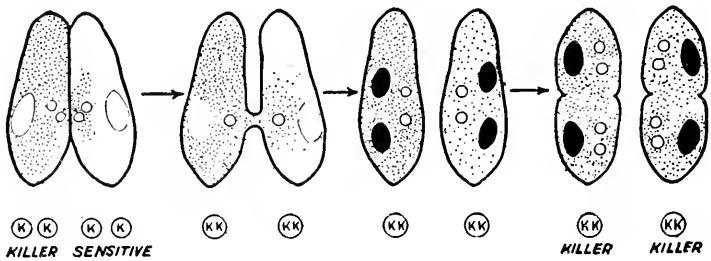


FIGURE 18.4

Transfer of kappa when conjugation lasts abnormally long. The rate of multiplication of the transferred kappa has been exaggerated for diagrammatic purposes. (From Beadle 1948, after Sonneborn and others.)

end of the range at which infection cannot be definitely demonstrated to occur, and the plasmagenes appear to be normal constituents of the organism. Particles coming at the latter end of the range may be considered as true plasmagenes. Most of the cases known are from the plant world (reviewed in Caspari 1948). Perhaps the best investigated are the factors which are clearly inherited through the cytoplasm in crosses between different races of the willow herb, *Epilobium* (Michaelis 1951). Another example is provided by the cytoplasmic factors causing male sterility in crosses between races of a number of different species of plants (e.g. flax, maize, etc.).

Evidence for such factors in higher animals is exceedingly rare. The case which perhaps seems most likely to fall into the category is that described by Laven (1953) in mosquitoes of the genus *Culex*. He made reciprocal crosses between a number of races of the species-group *Culex pipens*, and found that they fell into three groups; between the members

of a group, crossing led to offspring whichever race provided the female, while when the crosses were made between races of different groups, they only succeeded in one of the two possible ways. Thus the cross between the *O* and *H* races gave offspring only when the female belonged to the latter. Further analysis showed that the infertility of the opposite cross is due to a factor carried in the *H* sperm, which inhibits the development of the embryo when it gets into an egg with *O* cytoplasm. By backcrossing of the hybrid females to *O* males for several generations, one obtains males which will contain an almost completely *O* genotype; nevertheless they continue to give sperm which lead to a failure of development of the *O* eggs. It seems that this must be due to a cytoplasmic factor which came from the *H* female in the original interracial cross and which has been carried on by the eggs through subsequent generations, without being influenced by the increasing number of *O* genes. The nature of the factor is still obscure. It may be similar to the plasmagenes described in plants such as *Epilobium*. But its distribution is worthy of note. In *Culex* it distinguishes various geographical races; and within a race which has it, it is quite undetectable until an attempt is made at an interracial cross to a strain which lacks it. In another related genus, a similar factor, or perhaps the same, distinguishes the two species *Aedes aegypti* and *A. albopictus*; and in *Aedes scutellaris* there is a distinction between two local races similar to that in *Culex*. This suggests that we are dealing with a phenomenon which might be compared to the acclimatisation of some local races to a virus, rather than with a situation which can assist us to understand the origin of tissue differentiation. There are in the literature a few other cases of persistent cytoplasmic differences between local races (cf. Goldschmidt 1938 on *Lymantria*) but these require further investigation.

None of the entities in this category of true plasmagenes can yet be seen and there is no direct evidence as to the size of particle involved. The indirect evidence, chiefly from the type of physiological effect which they produce, is usually held to suggest that they are bodies of a gene-like order of complexity. It is not impossible, however, that in the future some of them may turn out to be simpler than has been previously thought.

3. *Visible cytoplasmic particles with genetic continuity*

In many Protozoa, self-duplicating cytoplasmic particles can be seen fairly easily in microscopical preparations stained in the appropriate way (by silver impregnation, for example). There are, for instance, the granules which lie at the base of the cilia with which the body of a ciliate is covered (Fauré-Fremiet 1948, Lwoff 1949, 1950, Weisz 1951). These so-called

kinetosomes lie in rows, and along the side of each row is a thread, the kinetodesma, the whole complex being known as a kinety (Fig. 18.5). It can be shown both by experiment and observation that kineties develop only out of pre-existing parts of kineties and they therefore possess at least one type of genetic continuity.

These kineties are of undoubted morphogenetic importance. In fact the structure of a ciliate, which may be quite complicated, is in the main

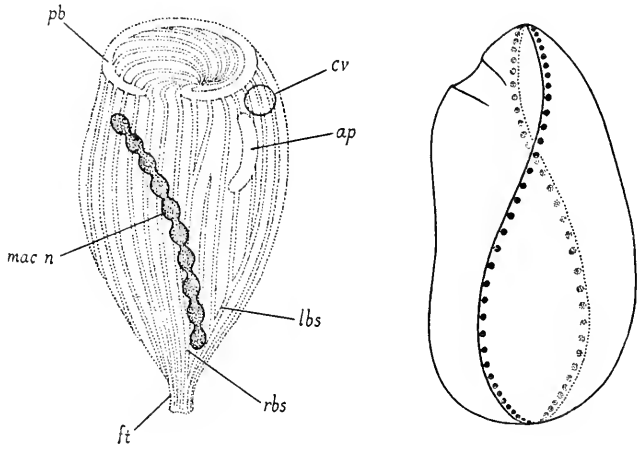


FIGURE 18.5

On the left, a drawing of Stentor: *mac.n.*, the nodular macronucleus; *pb*, peristome band; *cv*, contractile vacuole; *ap*, the adoral zone, from which the new peristome band originates in regeneration; *ft*, foot; *lbs* and *rbs* left and right sides of the ramifying zone, from which new kineties are formed. (Modified from Weisz 1951.) On the right, diagram of a ciliate, showing one kinety (composed of kinetodesma and kinetosomes) on the near side, and a similar one seen by transparency on the far side. (After Faure-Fremiet 1950.)

a matter of local differentiation of the cuticular layer or ectoplasm, and this can be shown to depend on the activities of the kineties. The evidence for this comes largely from observation of the process by which a single individual becomes reorganised into two at the time of cell-division, and from studies of the processes of regeneration of a whole individual from fragments. In both cases it is clear that the kineties are reorganised first, and only later produce the more obvious morphological structures such as the gullet, flagellae, cirri, trichocysts, etc. The kineties associated with these various organs do not, however, become fully determined, so as to possess only one specific character; thus a kinety originally associated

with a single cilium may, during regeneration, take part in the formation of a gullet or contractile vacuole or any other organ. The kineties are in fact organised in relation to one another in a 'gradient-field', somewhat reminiscent of that seen in Platyhelminthes, for instance. Thus there is a leading kinety (associated usually with the mouth), to which the rest are subordinated as the hind parts of a flatworm are subordinated to the head.

The fact that one and the same kinety may, under the conditions of regeneration or reorganisation, produce different structures, must mean that the effect of the kinety depends on the properties of the cytoplasm with which it is in contact. The situation is comparable with that which we have discussed in relation to the nuclear genes; the kinetosome may continue to duplicate itself identically but at the same time interact with the local cytoplasm, producing different active products according to the raw materials or other substances available. The organisation of the kineties into a gradient field is presumably brought about by the leading kineties affecting the cytoplasm in some way which diffuses outwards and influences the activities of the subordinated kineties.

Besides these mutual interactions between the various cytoplasmic particles, there are very interesting relations between the particles and the nuclei. Weisz (1951) has studied the matter in the particularly favourable case of *Stentor*. This is a large sessile ciliate, which possesses not only a micronucleus but also a large macronucleus which consists of a string of swollen nodes connected by a much thinner strand. The micronucleus seems to be concerned solely with sexual reproduction, and a *Stentor* from which it is missing can live for many generations of vegetative fission, and regenerate quite adequately (in some other ciliates the micronucleus is necessary for regeneration). These processes are, in fact, under the control of the macronucleus. The genetic constitution of this is not known with any certainty, but it seems likely that it is to be considered as a highly polyploid nucleus in which the original set of chromosomes has been multiplied many times; probably each node contains at least one diploid set of chromosomes and possibly more (Fig. 18.5).

During fission of a *Stentor* into two daughter individuals, the macronucleus also undergoes reorganisation, and in the period shortly after this it is found that all the nodes are equivalent, in the sense that any one of them is sufficient to make possible the regeneration of a whole individual. Later in the life-cycle, as the time approaches for the next fission, this is no longer the case. If at this stage a fragment is cut off a *Stentor* in such a way as to include only a posterior node, regeneration is not complete and a mouth is not formed. This is so even if the cytoplasm comes from an anterior region and is known to be capable of carrying out a complete

regeneration when provided with an anterior node. Further, if all but the posterior node is removed from an intact individual, the mouth and other anterior parts degenerate and disappear.

There is, then, a gradual loss by the posterior nodes of 'potency' to mediate regeneration or to support differentiated structures. This loss appears to be caused by the kineties of this region of the body, since if anterior nodes are forced into the posterior they soon lose their power to support full regeneration. We are therefore confronted with a two-way interaction between the cytoplasmic particles and the macronucleus; (1) the kineties influence the nearby parts of the nucleus, causing the posterior nodes to lose 'potency'; (2) the macronucleus controls the morphogenetic activity of the kineties, so that the mouth and other anterior organs cannot be formed in the absence of a fully potent nuclear node. It would seem, therefore, that the kineties cannot maintain their specific character, or at least cannot continue to produce their specific effect, without the collaboration of nuclear factors; their autonomy over against the nucleus is by no means complete.

4. *Gene-initiated plasmagenes*

In contrast to the preceding categories there are a group of factors, which are also often considered to be plasmagenes, and which are characterised by the fact that they can arise anew within cells from which they were originally absent. Their initiation seems in all cases to depend on the functioning of corresponding genes in the nucleus and is impossible if the effective gene is absent. Other conditions of an environmental kind are usually necessary to bring the gene into play and cause it to produce the cytoplasmic factor.

In *Paramecium*, besides the kappa particles which have already been described, there are cytoplasmic determinants of certain antigenic properties (Sonneborn and Beale 1949, Beale 1951, 1952, 1954). We have already (p. 359) referred to these in connection with the cytoplasmic control of gene activity and they also illustrate other points which may be relevant. Thus the cytoplasmic determinants (which are presumably particles, though they have not yet been seen) are under close control by genes. A given type of determinant cannot persist indefinitely in a cell from which the corresponding gene is absent; and in this their behaviour is perhaps similar to that of the *Stentor* kineties. Further, the genes in the *Paramecium* nucleus appear to be able to bring into existence the cytoplasmic determinants which correspond to them, even if there were previously no representatives of this particular type in the cell. Thus if *Paramecia* are kept at 29–33° C. they will develop one of the *D* antigens (depending on

which of the *D* alleles is present in the nucleus). If the strain is then transferred to 18°, the *D* antigen will eventually disappear, and an *S* antigen (corresponding to the *S* allele present) will take its place. It is, of course, difficult to be certain that there was absolutely no *S* antigen in the cells when they were originally kept at 29–33°, but it certainly seems probable that this was the case, and that the cytoplasmic determinants producing the *S* antigens have been formed anew under the influence of the *S* gene when it became activated by the effect of the lower temperature. These antigen-producing determinants therefore seem to provide examples of 'gene-initiated plasmagenes'.

Another important case is that described by Billingham and Medawar (1948, 1950, Medawar 1947, Fig. 18.6). If a patch of black skin from a spotted guinea-pig is transplanted into a white area, it is found that pigmentation spreads out from the graft into the surrounding area, forming a sort of halo. In mammalian skin, pigment is formed as granules in the cytoplasm of a system of highly branched dendritic cells, the melanocytes, which are present even in white areas, although there the pigment-forming system is inoperative. Billingham and Medawar argue that the spreading of pigmentation which they have studied is due to the 'infection'

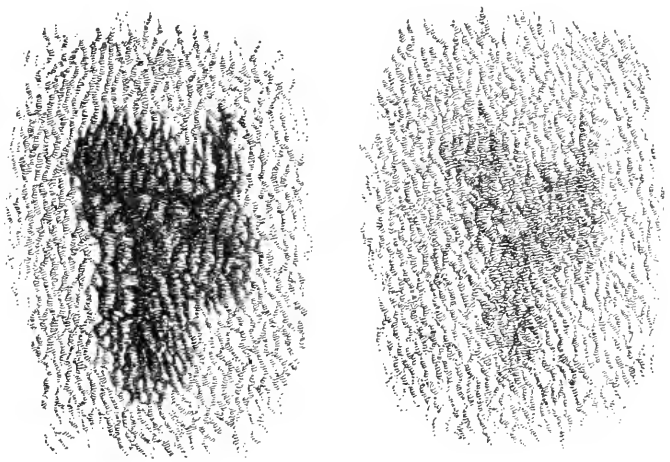


FIGURE 18.6

On the left, a shaved area of skin on a guinea-pig, into which a graft of black skin had been made 50 days previously: the black patch is due to induced pigmentation, most if not all of the grafted cells having died. On the right is shown the condition 20 days after an immunising graft from the same original donor was made; the pigmentation is very largely bleached away, although some traces remain in the centre of the area. (After Billingham and Medawar 1950.)

of the melanocytes surrounding the graft by a virus- or plasmagene-like particle from the pigmented cells of the transplant. This particle must be capable both of self-duplication and of causing the host cell to produce pigment; possibly it may be the pigment-forming particle itself, in which case these two actions would amount to the same thing.

There are two alternative hypotheses to be eliminated before this theory can be accepted. On the one hand, the spreading of dark pigmentation around a graft might be due to the migration of actual pigmentary cells rather than of intra-cellular particles. Such migration certainly occurs in embryonic grafts of pigmentary cells in birds, but Billingham and Medawar argue that it does not account for all the phenomena observed in mammals, if indeed it occurs at all. In particular, it is difficult to reconcile with the fact that if a graft of, say, black skin from the ear is grafted onto the sole of the foot, and pigmentation spreads around it, this blackened area will have the characteristics usually found in sole-of-foot epidermis and not that of the ear-skin which initiated it. But although this makes it difficult to suppose that the spread of pigmentation is due to the migration of actual ear-skin cells, it suggests the other of the alternatives to the plasmagene hypothesis, namely that we are dealing merely with a serial evocation, in which some substance of low molecular weight diffuses from a dark cell to the neighbouring unpigmented one and sets going within it an already present pigment-producing system. There are, however, phenomena which this possibility cannot easily explain. For instance, if a graft is made of black skin from guinea-pig *A* onto guinea-pig *B*, the halo of pigment formed round it retains the immunological specificity of individual *A*. If another graft of *A*'s skin is made onto *B*, it will set up an immunological reaction and as a consequence of this the pigment particles disappear from the zone of pigment spread around the original graft, although the dendritic cells themselves do not seem to be adversely affected. Billingham and Medawar, on these and other grounds, argue that we must be dealing with a particle large and complex enough to carry the immunological specificity of the individual from which it originated, and capable both of spreading from cell to cell of the dendritic system and of identical self-duplication.

This case is of great interest and theoretical importance, since it is one of the very few examples of an alleged plasmagene in a higher animal which has been thoroughly studied in recent years, and in which there is no question of our being deceived by an extraneous virus. There are several further points to notice about it. First is the rather surprising fact that the 'plasmagene' carries the immunological specificity of the individual in which it arises, which makes it extremely difficult to transmit

from individual to individual (although this can be achieved if the graft is small enough not to provoke an intense anti-body formation by the host). In this it is very different from most viruses. Secondly, the plasmagene does not possess complete genetic continuity, but must arise anew during the development of the melanocytes from the neural crest; presumably the initiation is gene-controlled, although the difficulty of inter-individual transplantation makes it difficult to prove this formally. Again, one must remember that the pigment-forming cells are peculiarly adapted to make contact with their neighbours, and are known to pass pigment granules into cells, e.g. in the hair follicles, which are themselves incapable of manufacturing pigment. Thus the situation which enables us to recognise the existence of these plasmagenes by their transmission from cell to cell is a very unusual one. One might deduce from this that many such particles might exist in other cells, without our being able to detect them; equally one might argue that it is only because the melanocytes provide this peculiar mechanism of passing granules into contiguous cells that particles which can multiply and preserve their genetic character have been evolved to take advantage of it. Finally, the relations between the particles and the genetic factors in the nucleus remain obscure because we do not understand what genetic difference, if any, exists between a white and a dark area in a piebald guinea-pig; both areas are, of course, derived from a single fertilised egg, and probably both contain exactly the same genes, although some authors have suggested that the piebald pattern is due to mutation of a colour-controlling gene in some of the somatic cells. It remains obscure, therefore, whether, when the pigment-forming plasmagene is passed into a white cell and proceeds to multiply there, it is aided in doing so by the gene which initiated it, or whether that gene has mutated to an inactive form and the plasmagene is wholly responsible for the maintenance of its genetic character.

Some suggestive but indirect evidence for the existence of cytoplasmic plasmagene-like particles in normal cells can be found in some of the studies which have been made on tumour-inducing 'agents' of the kind which are often classed as viruses. These agents seem to be particulate in nature, and when suspensions of the particles are injected into appropriate healthy cells, they can multiply and cause the cells to develop into tumours. One important point in the present connection is that particles extremely similar to those of the tumour-virus can also be found in normal healthy cells; they are in fact the microsomes (Claude 1940). Further, when normal cells are acted on by certain carcinogenic chemical substances, the tumours which are induced are found to contain cytoplasmic particles capable of acting as tumour-viruses; and it is certainly simplest to suppose that these

have originated by a comparatively slight alteration in the normal cell particles, to which one would therefore have to attribute a capacity for self-duplication. Finally, the tumour-viruses sometimes show extreme specificity in the type of tissue they can infect; indeed Rose (1952*b*) has shown that a frog virus may be so specific that it travels through the body and settles in only one particular region of the skeleton (cf. the reference to regional specificity on p. 308). Now Rose has found that if the agent can be caused to grow in an unusual tissue, it may acquire a new specificity from it; its original specificity is sometimes retained, sometimes lost. He suggests that the new character may be picked up by an interchange process, akin to bacterial 'crossing-over' or 'transformation', with cytoplasmic constituents of the normal cells which are essentially similar to the virus particles (but cf. Luria 1953). The suggestion is interesting, and considerable developments may be expected in this field; but at present the whole of this group of phenomena is so little understood that it seems dangerous to use it as a basis for a general theory of differentiation. For instance, according to Rose these tumour-inducing agents seem, after passage through a number of different hosts, to lose nearly all their tissue or even species specificity, which makes one wonder whether they may not be damaged forms of the normal cell microsomes, the damage being of such a kind as to render them insensitive to the normal control of the nucleus; if this were so, they could not be taken as evidence that the cytoplasm of a healthy cell contains particles which are independent of the nucleus.

Another type of phenomenon, which may involve the activity of plasmagenes, is that known as enzymatic adaptation (Reviews: Monod 1947, 1950, Monod and Cohn 1952, Spiegelman 1950, Gale and Davies 1953). There is abundant evidence which strongly suggests (though perhaps it does not completely prove) that in bacteria, yeasts and other lowly organisms suitable for such studies, the formation of most enzymes attacking exogenous substrates is specifically increased by the presence of the substrate and in fact hardly occurs at all in its absence. The formation of the adaptative enzymes involves the synthesis of protein, and the physiology of the process has been studied by several authors (e.g. Spiegelman and Sussman 1952). The formation of an adaptive enzyme in the presence of any particular substrate requires the activity of a corresponding nuclear gene, and we therefore have here a good example of the influence of substrates on the activities of genes. But it has been suggested that something more is involved. Lindegren and Spiegelman (*Cold Spring Harbor Symp.* 1946) originally put forward the hypothesis that the production of the adaptive enzyme was a property of a cytoplasmic plasmagene,

which, in the presence of the substrate, could persist indefinitely even if the corresponding gene had been removed from the cell by crossing. Their original evidence, obtained in yeasts, was later shown to be inadequate, but there still remains some evidence which may indicate that such plasmagenes exist (Spiegelman 1951).

Ephrussi (1953), (Ephrussi and Hottinguer 1951) has also found evidence which suggests the existence of cytoplasmic self-duplicating particles concerned with enzymatic adaptation. In his case, yeast cells cultivated in the presence of the nuclear poison acriflavine are shown often to give rise to colonies which grow abnormally slowly and in which the cells have lost certain respiratory enzymes and have an abnormal cytochrome system. The change is quite stable through many generations of vegetative division. Crossing experiments show that it is immediately dependent on a cytoplasmic, not on a nuclear, factor; but again further investigation has demonstrated that the plasmagene concerned is itself under the eventual control of a gene. The enzyme changes induced by the acriflavine are not themselves adaptive, but they are closely similar to undoubtedly adaptive changes which yeast cells exhibit when cultivated in the absence of oxygen.

It is questionable whether it is really appropriate to employ the word plasmagenes for any of the gene-initiated factors considered in this section. The character they share with the true plasmagenes is a certain ability to multiply in the cytoplasm. It is not clear, however, that any case is known in which a gene-initiated cytoplasmic factor acquires complete autonomy in its powers of reproduction. Certainly the *Paramecium* antigen determinants can only persist for a very limited period after the removal of the gene. The situation of the factors studied by Billingham and Medawar is obscure, since in the piebald guinea-pigs they studied, the originally colourless cells into which the factor passes probably possess the same genotypic constitution as the coloured cells out of which it comes, the difference between the cells being one which arises during differentiation rather than of a truly genetic nature. Beale (1954), who has studied these phenomena as closely as anyone, has recently expressed a lack of satisfaction with the term plasmagene for such factors. Haldane (1954) is apparently of a similar opinion and has suggested calling them mnemons. For convenience in the present discussion, however, I shall continue to refer to them as gene-initiated plasmagenes.

5. *The role of plasmagenes in differentiation*

It will be noticed that the overwhelming majority of the evidence for the existence of plasmagenes come from studies on micro-organisms. It

might be, however, that this is caused not by their rarity in other forms but by factors which make their detection particularly difficult. It is clear, for instance, that if plasmagenes were to play an important part in the differentiation of multi-cellular organisms, they could not in general be capable of easy infective transmission from one cell to another, since that would lead to an intermingling of different organs or types of tissue which should remain separate. Thus we cannot expect to find many cases similar to that of Billingham and Medawar, even if factors of an essentially similar nature are widespread. It is necessary, therefore, to approach the matter to some extent from an *a priori* point of view to try to determine how far plasmagene-like factors could fit in to the mechanisms of differentiation in so far as we understand them at present.

It is clear that the exogenous factors mentioned under group (1) above do not come into the question. In the examples of the true plasmagenes mentioned in group (2), the cytoplasmic determinant is a part of the general genetic constitution of the organism and no more directly related to the regionalisation of its various parts than are the nuclear genes. It is, however, possible to imagine that the cytoplasm of the egg of a given species might contain a number of different true plasmagenes localised in various regions. Each region of the egg would then contain characteristic cytoplasmic factors endowed with genetic continuity which might determine the nature of the organs which develop out of it. Such localised plasmagenes would, in fact, be the same thing as used to be referred to at the beginning of this century as organ-forming substances. Now there is no doubt that in many eggs different regions of the cytoplasm have different properties. The regions concerned are nowadays referred to as ooplasms, and opinion has rather moved against attributing their properties to the presence of substances which are autonomous over against the nucleus.

The arguments which have swayed opinion against the old idea of organ-forming substances are numerous. One is that the evidence suggests that the ooplasms are only effective when they are able to interact with the nuclei. For instance, the cytoplasmic formation centre in the posterior of an insect egg only becomes active when nuclei reach it (p. 125); the same is true of the grey crescent ooplasm of the amphibian egg (p. 149). Again, differentiation from the egg to the final form takes place in a series of steps. It does not look as though we are dealing merely with the sorting out of a number of factors which from the beginning preserve their character unchanged, but rather as if development consists of a series of reactions during which the constituents of the system change continuously until the final condition is gradually built up. We are already faced with the difficulty of accounting for this progressive series of changes in a system

one of whose major components consists of genes which we believe to retain their identity throughout. The difficulty is only made the greater if we have to suppose that the major factors in the cytoplasm also retain their identity.

As a third argument, one may point to the fact that the localisation of different organs within the developing body may often be altered by factors which operate after the segregation of plasmagenes in the egg cytoplasm must have been completed. For instance, one might be tempted to attribute the localisation of the organs in a developing *Drosophila* to the segregation of organ-forming substances or plasmagenes in the eggs, which are known to belong to the mosaic type; yet we have seen (p. 141) that environmental treatments applied many hours after fertilisation can divert the differentiation of particular regions into abnormal paths.

The mere fact that a gene, like aristopaedia, can cause a mass of tissue which should normally develop into an antenna to develop into a leg instead, shows that even if we try to attribute the major process of differentiation to plasmagene-like bodies, these cannot be autonomous in their properties but must be highly susceptible to modifications caused by interaction with genes. Finally, it would be still more difficult for a hypothesis which attributed differentiation to be activities of autonomous plasmagenes to account for metaplasia, which, though rare, does seem to occur (p. 308).

It appears, therefore, that the postulation of true plasmagenes as organ-forming substances in the cytoplasm of the egg does not materially simplify the theoretical task of accounting for the phenomena of differentiation. That does not necessarily mean, of course, that such bodies do not or cannot exist; we should have to take account of them if there was unequivocal evidence for the existence in the eggs of multi-cellular animals of cytoplasmic factors which had genetic continuity independently of the nucleus. As yet there seems to be no compelling evidence to this effect. Indeed, attempts to assess the autonomy of cytoplasmic factors in the egg over against the nucleus have been few and far between. The studies with hybrid merogons in Amphibia (p. 358) are perhaps the most promising. The facts there can probably all be accounted for in terms of a mere persistence of cytoplasmic character, without the need to postulate that the cytoplasmic factors can reproduce while retaining their specific nature. The case which argues most strongly in the opposite direction is perhaps that of Hadorn (1936), who found that epidermis derived from *Triturus palmatus* cytoplasm fertilised by *T. cristatus* sperm developed the typical characteristics of *palmatus* as late as after metamorphosis. This may indeed be evidence of the existence of a true plasmagene, but it might equally be

the result of a plasmagene initiated in the egg cytoplasm by the maternal genes during the maturation of the egg. One may conclude that there is hardly any evidence that plasmagenes with complete autonomous genetic continuity exist in metazoan eggs, and that it seems most improbable that the major phenomena of differentiation can be attributed to them.

The same conclusion applies even more forcibly to the plasmagenes of category (3), namely microscopically visible cytoplasmic particles with genetic continuity. These certainly occur in certain special cases, as for instance in ciliates, but in general the histological evidence makes it clear that differentiation does not consist to any large extent of the mere sorting out of the already existing visible particles in the egg cytoplasm. Such particles probably play an important part in development, but not by the mere retention of their original characteristics.

The situation is rather different when we turn to the fourth category, that of gene-initiated plasmagenes. If these were to play an important part in development we should have to imagine that the various ooplasms of the egg differentially excite the nuclei which enter them; that the particular genes which are activated in a given region then cause the appearance of cytoplasmic factors, and that these factors, when they have appeared, show a certain degree of autonomy, being able to reproduce for a short time with repetition of their character even if the nucleus is removed or changed. If one supposes that, once they have been formed, the autonomy of the plasmagenes is complete, this suggestion would come up against the same difficulties as confronted the hypothesis of organ-forming substances in accounting for the sequential character of differentiation and phenomena such as the metaplasia of retinal cells into lens in Wolffian regeneration. We have seen, however, that in the best-studied examples of gene-initiated plasmagenes the autonomy is by no means complete. If one waters it down sufficiently, the difficulties which have just been mentioned could be overcome.

The hypothesis would then amount to the suggestion that during differentiation the genes cause the appearance in the cytoplasm of bodies with a certain limited amount of autonomy. There seems nothing impossible, or even very difficult, in such a suggestion. As was pointed out earlier (p. 212), Brachet (1944, 1952*a*) has argued with considerable persuasiveness for the importance of the ultra-centrifugable ribose-nucleic-acid-containing microsomes, and he does not hesitate to refer to these as plasmagene-like in character. The problem that still remains at issue is how far these particles, once their character has been determined, become independent of the nucleus. Only the transplantation either of the nuclei or of the particles from one type of differentiating cell to another can settle the matter con-

clusively. There would be nothing surprising if experiment eventually showed that, in cells which are more or less completely determined and are in process of producing their final cytoplasmic constituents, the cytoplasm is able to carry on synthesising these nearly independently of the nucleus. However, such a fact does not yet seem to have been demonstrated. Whether, if it were, we should be justified in speaking of the effectiveness of plasmagenes in differentiation would be largely a matter of definition; it would depend on whether we are satisfied that such gene-initiated cytoplasmic factors of limited autonomy are comparable to plasmagenes of the more completely autonomous kind.

SUGGESTED READING

Darlington 1944, Ephrussi 1953, Haldane 1954, Chapter 7, Medawar 1947, Monod 1947, Sonneborn 1951*a* or *b*, Weisz 1951.

CHAPTER XIX

THE DIFFERENTIATING SYSTEM

IT IS now time to try to formulate a general theory of differentiation based on the various factors which have been discussed above.

Perhaps the first problem which should be considered is the development of differences between the various regions of the embryo. In the scheme of intra-cellular reactions which was suggested earlier (see Fig. 16.1, p. 349), the nature of the cytoplasm affects the course of events at two different stages; on the one hand it provides the raw materials for gene activity and may thus differentially activate or inhibit different genes, and on the other it has the same relationship to the immediate gene products (and plasmagenes, if any). It is therefore easy to see how the constitution of the cytoplasm could set going a number of dissimilar processes of differentiation. In fact, it would be quite possible for this to occur through the interaction of the cytoplasm with the gene products, even if the activity of the genes themselves was exactly the same in all cells; but as we have seen there is actual evidence of nuclear differentiation in the various tissues, and there seems no reason to doubt that both the possible influences of the cytoplasm—on genes and on gene products—are effectively in operation.

The next point is the existence of distinct alternative pathways of chemical change, leading to the production of a finite number of definite tissues; and the peculiar mixture of permanence and lability revealed in the phenomena of determination and modulation. The explanation for the distinctness of the developmental paths can probably be found in the nature of the cytoplasmic influences on the genes and gene products. All the different genes are made out of similar building blocks, i.e. the amino-acids and peptides which go to form the protein, and the nucleotides which form the nucleic acids. The same situation holds for the gene products. Thus we must suppose that the various genes (and gene products) will compete with one another for the available raw materials. There may also be other types of competition; for instance a high concentration of one gene product *A* may inhibit the formation of another *B*, and so on. There will therefore be a situation of 'competitive interaction' in the formation of the gene products and another in their production of cytoplasmic substances.

Now in a system consisting of a mixture of raw materials for which several synthetic processes are competing, situations can easily arise in which a slight change in initial conditions will have a great effect on the final state; in fact, such systems will often be such that there are only a limited number of final states to which they can attain, the choice between one end-state or another depending on the concentrations of substances present at the beginning (Waddington 1948*b*, 1954). To take a simple example of what may happen, consider two substances P and Q, which are being formed out of the raw materials A, B, and C, for the supplies for which they compete. Suppose competition occurs because P is formed from A and B, while Q is formed from B and C. Again for the sake of simplicity, let the reaction constants be the same for the two syntheses, as shown in Fig. 19.1; and let A, B and C diffuse into the system at rates proportional to the difference in their concentration inside (A, B, C) and outside (a, b, c), while P and Q are removed at rate k_3. Finally, let us suppose that the coupling of A and B to form P, and of B and C to form Q, are autocatalytic processes, i.e. are speeded up by the presence of already-formed P and Q. This is a simple form of a 'feed-back' mechanism. The equations for the rates of change of the various components will be

$$\frac{dA}{dt} = k(a - A) - k_1PAB + k_2P^2$$

$$\frac{dB}{dt} = k(b - B) - k_1PAB + k_2P^2 - k_1QBC + k_2Q^2$$

$$\frac{dC}{dt} = k(c - C) - k_1QBC + k_2Q^2$$

$$\frac{dP}{dt} = k_1PAB - k_2P^2 - k_3P$$

$$\frac{dQ}{dt} = k_1QBC - k_2Q^2 - k_3Q.$$

At the steady state, we find a relation between P and Q of the form

$$(kk_2c + k^2{}_3)P = (kk_2a + k^2{}_3)Q + kk_3(a - c) \quad . \quad . \quad . \quad (1)$$

Now if k_3 is small compared with k (i.e. diffusion out of the system is slower than diffusion in), then we can neglect its higher powers, and we find

$$P = \frac{a}{c}Q + \frac{k_3}{k_2}\frac{a - c}{c} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Thus if initially in a certain region the supply of A is increased relative to that of C, we find that P will be increased relative to Q by something more than a proportionate amount, the excess being expressed by the last term on the right. And if the rate of removal of P, (that is k_3), is greater than the rate at which P breaks down again into A and B, (that is k_2), this excess can be considerable. We can also see from expression (2) that the exaggeration will be the more important the smaller the absolute values of P and Q; and these will also be reduced if k_3 is fairly large, so that P and Q are rapidly removed.

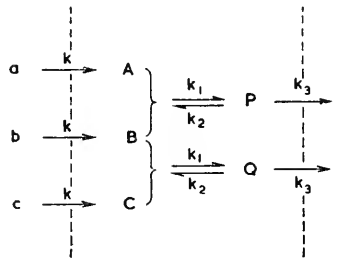


FIGURE 19.1

A system of competing chemical reactions, by which substances A, B and C become converted into P or Q. See text. (From Waddington 1954*a*.)

Without going into further details, we can see that if two autocatalytic processes compete for raw materials, we may under some conditions find that an initial change in the supply of the materials produces an exaggerated effect on the steady-state concentrations of the synthesised products, and thus on the rates at which these products can be made available outside the system.

If we suppose that A, B, and C are the raw materials out of which two genes manufacture their immediate products P and Q, we have now developed a picture by means of which we can see how a change in the concentrations of these raw materials leads to exaggerated differences in the rate at which P and Q are passed out of the nucleus into the cytoplasm.

It is, however, by no means the only model which might be appropriate. As Delbrück (1949) has suggested, there might be direct interactions between the two synthetic processes. These are perhaps most simply formulated by supposing that P is destroyed at some rate proportional to the concentration of Q (and vice versa). The equations for dP/dt and dQ/dt will then contain terms in PQ. If we regard the system as closed,

rather than open as was the system discussed above, and if the supplies of raw materials are taken as constant, the equations which result are of the same type as those which arise in the study of the growth of two populations of animals which compete with one another for a limited food supply. Lotka (1934) had discussed the relatively simple situation of two populations (or substances) for which the equations take the form

$$\frac{dP}{dt} = m_p P - k_p P^2 - k_{pq} PQ$$

$$\frac{dQ}{dt} = m_p Q - k_p Q^2 - k_{pq} PQ.$$

He shows that according as $m_p k_q$ is greater or less than $m_q k_{pq}$, and $m_p k_{qp}$ greater or less than $m_q k_p$, so the final state of the system is either wholly P or wholly Q, or a certain fixed ratio between them, or finally the system is one which will finish up either entirely P or entirely Q according to the initial concentrations of these substances.

Kostitzin (1937) has also discussed shortly the more general case in which there are many competing and interacting substances (or populations), so that we have a large series of simultaneous differential equations, each containing terms of the second order, such as P^2 or PQ, etc. He shows that such a system may be expected to exhibit a number of alternative steady states, some at least of which are likely to be stable, and that the particular one which the system actually attains will in many cases depend on the initial conditions.

Competitive interactions are not only almost necessary consequences of the nature of the situation, but there is definite and direct evidence for their existence. Perhaps the most striking is that produced by Spiegelman (1948, 1950) from the study of adaptive enzyme formation in yeasts. If a yeast is grown in the presence of two substrates, for neither of which it originally possesses the appropriate enzyme, it will gradually manufacture the suitable adaptive enzymes, which are of course protein in nature. If the supplies of nitrogen are restricted, these two proteins enter into obvious competition with one another for it, one or both of the enzymes being formed more slowly when the yeast is adapting to two substrates simultaneously than when there is only one new substrate (Fig. 19.2). Spiegelman also shows that the systems producing the adaptive enzymes are self-reinforcing or 'autocatalytic'; and the way in which a muscle cell, for instance, fills with myosin, suggests that the same is true of the formation of many cytoplasmic proteins. We have thus all the components

required for a system which will tend towards a limited number of alternative end states.

It is not so easy to give an actual example of a system in which the alternative end states depend on specific inhibitions, of the kind postulated by Delbrück. However, Horowitz (1951) has mentioned a case, which, although it is still somewhat hypothetical, may serve as an example of the kind of situation which it would be most desirable to analyse. When the mould Neurospora is grown in sulphur-deficient media it develops

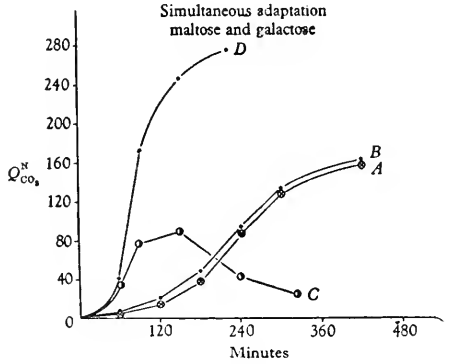


FIGURE 19.2

Simultaneous adaptation of yeast to maltose and galactose. The curve *B* shows the activity in fermenting galactose, when that is the only sugar present; *D* is the corresponding curve for maltose. When both sugars are present, the splitting of galactose is not much affected (*A*) but the growth in maltose-splitting activity is competitively inhibited (*C*). (After Speigelman 1948.)

the enzyme tyrosinase, and tends to become blackened with melanin pigment if any suitable substrate is available. It seems probable that in fact this enzyme is also produced in normal media containing sulphur, but that in them some sulphur-containing compound which inhibits tyrosinase activity is also formed. Horowitz asks what would happen if one of the products of tyrosinase activity could combine with the inhibitor and destroy it, or in some other way prevent its appearance. Then, he suggests, if a mould were grown for some time in a sulphur-deficient medium, it would acquire a high content of tyrosinase, and on transference to a normal medium this enzyme would continue to be active unless or until the production of inhibitor could overtake its inactivation by the tyrosinase-system. We might then have a very simple example of a system

with two alternative types of differentiation (with and without active tyrosinase and formation of melanin pigment), the choice between which would be dependent on the previous history of the strain (whether it had spent a period in a sulphur-deficient medium).

There appears therefore to be no difficulty in accounting in one or other of these ways for the existence of distinct alternative paths of differentiation. Within a given path, there are two types of phenomenon for which we have to provide an explanation. The first is the existence of a range of variation of the kind which is exhibited in 'field' processes. For instance, if some cells in the limb region enter on the path of development leading to cartilage and bone formation, they may have the character of the femur or, on the other hand, of some other part of the limb skeleton. A somewhat similar phenomenon is that of 'modulation'. In this case a given tissue assumes a range of histological forms in dependence on the nature of its environment, but throughout all such changes retains its essential character unimpaired. Presumably in both cases the variations are basically quantitative in nature, and indicate that the concentrations of the reacting gene-products and cytoplasmic substances can take a certain range of values while still remaining within one and the same alternative path of development.

In contrast to such flexibility of behaviour is the essential permanence which is alluded to by speaking of tissues as 'determined'. When tissues are modulated by some external influence, they nevertheless retain their original character and can re-express it when suitable conditions arise. Can the hypothesis which attributes determination to competitive interaction provide a satisfactory explanation of this? The question has been little studied, either theoretically or practically. There is no doubt that one can invent systems of competitive interaction which would, if they actually existed, make differentiation very difficult to reverse. The mere co-existence of numerous interacting substances would almost suffice. Evolutionary changes hardly ever in practice get reversed, just because they depend on such numerous gene changes that it is extremely improbable that all the reversals will happen simultaneously. In the same way, a highly complex system of competitive interactions would scarcely ever be brought to retrace its steps. But that is a very generalised argument; and the occurrence of modulation, and of peculiar combinations of lability and fixity of character (such as the lability of avian epidermis which allows it to be induced to form a feather, combined with its fixity of tract-specificity, p. 259), make one wish for some rather more detailed understanding. This could probably come partly from a mathematical investigation of the theory of stability of competitively interacting systems.

Much could probably be learnt, also, from specially designed experiments on simple examples of them.

Until such time as further theoretical or experimental studies have been made of the properties of such systems, one is left with a certain freedom of choice as to what hypothesis seems adequate to explain the facts of determination and modulation. It may well be that there is no need to assume anything more than competitive interaction between autocatalytic processes leading from genes to gene products, and from the latter to the final cytoplasmic constituents.

Some authors, however, feel that a further factor tending towards persistence of character is required, and suggest that this can be found by invoking the presence of plasmagenes. This possibility was discussed in the last chapter, where the conclusion was reached that gene-initiated plasmagenes would seem the most likely kind to play a large role in development, but that although there is no doubt that the cytoplasm contains complex bodies of a roughly gene-like order, one requires more evidence of their independence of nuclear control before accepting them as plasmagenes. This additional evidence can, as far as one can see at present, be sought only in a further study of the properties of the microsomes to which Brachet has attached so much importance in the synthesis of proteins. Are they indeed the main site of protein production, as he suggests? And if so, have they in addition the property of multiplying and retaining their own specific character in some degree of independence of the genes? If so, one might allow that they were plasmagenes of one or other of the types indicated in Fig. 18.2. Really conclusive evidence on these points will, probably, only become available when the technical difficulties of transplanting microsomes from one cell to the other are overcome, and their degree of autonomy over against the nucleus can thus be investigated. In the meantime, the best evidence we have as to the developmental functions of microsomes comes from the phenomena of evocation.

In evocation we are undoubtedly confronted with a situation in which an environmental influence, impinging in the first place on the cytoplasm of the competent cell, causes it to adopt one or other of the alternative paths of development open to it. The fact that the reacting tissue retains its own specific characteristics—*T. alpestris* ectoderm forming typical *alpestris* neural tissue even if evocated by *T. cristatus* mesoderm—shows that the developmental paths are under genetic control and that the evocation involves the differential activation of a particular set of genes. The problem of the mechanism of evocation therefore becomes that of the nature of the influences which can activate or inhibit genes; that is to

say, which can produce crucial changes in the systems of competitive interactions. We know that the initial evocating stimulus may be comparatively slight, even a mere change in pH. We do not yet know on exactly what part of the cell system this obtains its effect. Weiss (1947, 1949*a*) has pointed out that an evocating stimulus might act at the cell surface, by causing the accumulation there of a particular molecular species, with a consequent depletion of the deeper parts of the cell and an alteration in the 'molecular ecology', or systems of competitive interaction (Fig. 19.3). There is, however, little convincing evidence that evocating actions take place primarily at the cell surface (cf. p. 213).

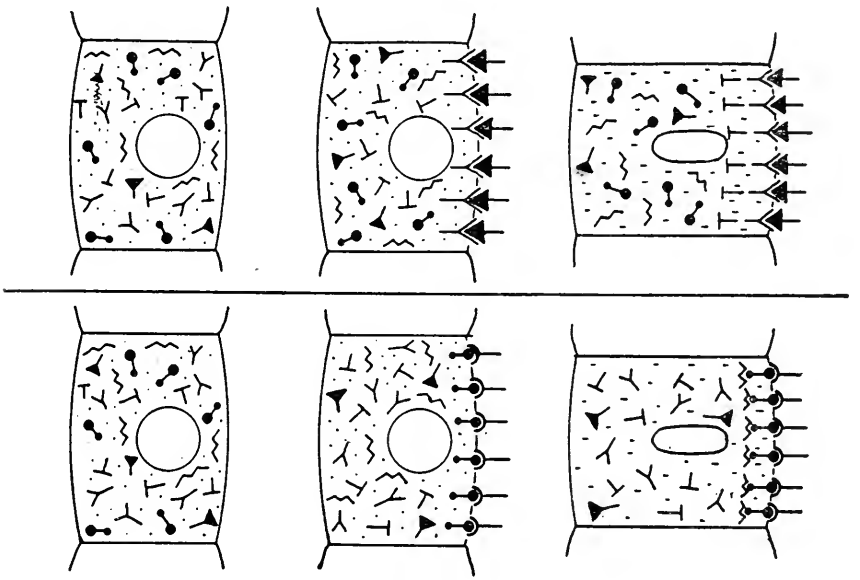


FIGURE 19.3

Diagram showing how two different substances (upper and lower rows) acting on the surface of a cell might attract to the surface different specific internal constituents, and thus cause progressive cellular differentiation. A conceivable mechanism for evocation. (From Weiss 1950*b*.)

Indeed the most critical evidence as to the location of evocator action speaks in the opposite sense. Waddington and Goodhart (1949) investigated the position taken up within the cell by the sterol-like hydrocarbons which are extremely active evocators. It was found that they are not absorbed on the surface or on the nucleus, but on lipo-protein granules (lipochondria) in the cytoplasm, which then break down to give microsomes.

This is the only case in which the location of an evocating substance within the cell has been verified; and the trail appears to lead to cytoplasmic particles and eventually to the microsomes. Brachet (1944, 1947, 1952) has advanced a number of lines of argument suggesting that these particles are intimately involved in evocation (p. 212). It may be that all or most of the many substances capable of evocating neural tissue act in the first place on these granules. We saw, however (p. 222), that the microsomes are only one element, though an important one, in the complex flux of cellular metabolism, and that one cannot consider the mechanism of evocation without taking account of gene action, protein synthesis, respiration, etc. The biochemical processes symbolised by the two cycles of reactions in Fig. 16.1, from the cytoplasm to the genes, and from the cytoplasm to the gene products, must actually involve all the basic metabolism of the cell. It is fruitless to envisage evocation as a special reaction, carried out by some particular part of the cell which can be, in theory, isolated from the general body of the living system. We must be prepared, therefore, to find that several different types of influence, impinging on the double cycle of cellular metabolism, may result in the same effect of swinging these cycles into one or other of the alternative modes open to them. Similarly, when we think of particular groups of genes being activated in different tissues, the cytoplasmic conditions responsible for this probably cannot be reduced to the mere concentration of certain inactive raw materials which lie quietly there for the genes to utilise. The 'substrates', for which we have envisaged the genes competing, will themselves in many cases be involved in active chemical processes of respiration, energy transfer, etc. The genes and gene products must be thought of as focal points in a continuously active and dynamic system.

SUGGESTED READING

Horowitz 1951, Lehmann 1950, Schultz 1952.

CHAPTER XX

INDIVIDUATION—THE FORMATION OF PATTERN AND SHAPE

DIFFERENTIATION does not consist merely in the production of new 'substances'—be they simple ones, such as pigments, or complex, such as the various types of tissue. We also have to consider the arrangement of these substances into definite relative positions, and, usually, the moulding of them into characteristic shapes. Development, in fact, produces not merely tissues but organs. It is to this type of phenomenon that the name 'individuation' has been given. It has, as has been implied above, two rather different aspects. On the one hand there is the question of the spatial distribution of the different substances. For instance, within the sheet of developing mesoderm a notochord develops in the dorsal mid-line, flanked on either side by somites, with the nephric mesoderm more laterally again and side-plate mesoderm outside that. We have earlier (p. 12) used the term 'regionalisation' to refer to the appearance of different parts within an originally uniform expanse of tissue. This is one of the aspects of individuation and before considering it further we should remind ourselves that regionalisation normally takes place so as to produce definite patterns of arrangement of the different parts. It is not adequate to picture it merely as a process by which a number of intermingled entities become sorted out into heaps of like components; we must add the fact that the heaps are mutually arranged in orderly patterns.

The other aspect of individuation is the formation of three-dimensional structures. For instance, the hollow sphere of the blastula undergoes the process of gastrulation and thus acquires a new and definite configuration; or again, a neural plate rolls up into a neural tube, which is characterised by the well-defined swellings of the brain vesicles, etc. All such processes are 'morphogenesis' in the strict sense, since that word really means the development of shape. The shapes of organs and of the body as a whole continue to change throughout most of life owing to the unequal growth of different parts. Such processes of relative growth have been considered in Chapter XIII; they may be considered as secondary morphogenesis. What we shall be concerned with in this chapter are the processes of primary morphogenesis, by which the original shape of the organ rudiments is first brought into being. (The distinction between these two categories of primary and secondary morphogenesis is not

sharp, but it is a useful rough classification and we shall see that in primary morphogenesis there are many factors which play a more important part than differences in growth rate.)

The two aspects of individuation—morphogenesis and pattern formation—are obviously closely connected with one another. It is hardly to be supposed that any complicated three-dimensional structure will develop unless the material out of which it is made has already developed a pattern of different properties in its various parts. Thus some degree of pattern formation probably always precedes any but the very simplest morphogenetic processes. Contrariwise it is to be expected that a developing pattern will be influenced by the shape of the area or mass in which it is forming and we shall find examples which demonstrate that this is the case. It is, however, helpful to use the distinction between pattern formation and morphogenesis as a means of arranging the subjects which require discussion into some sort of order. Moreover there is a certain difference in the kind of processes which must be involved in the two classes of phenomena. Pattern formation can, and frequently does, go on within a mass whose overall shape does not change. It requires the postulation of forces of an essentially chemical or physico-chemical order —diffusion, facilitated synthesis and the like. Morphogenesis, on the other hand, involves the actual movement of masses from one spatial position to another, and requires the intervention of physical forces such as those of surface tension, attraction, contraction, expansion, etc.

Pattern formation and morphogenesis are typical examples of field phenomena, since they involve processes which are both extended throughout a region of space and which also have a certain unity. As was suggested earlier (p. 23) such fields arise from the interaction of a number of different factors each of which is extended throughout the region involved. We cannot expect, therefore, to be able to attribute the formation of a pattern to the action of any one single factor, but must expect always to find that several different things are involved in it; and the same expectation of a multiplicity of causes rather than a single cause holds goods for morphogenesis.

Although pattern formation and morphogenesis occur in the differentiation of all organs and embryos yet there are not very many instances in which they have been closely studied as the main subjects of investigation, and we still know disappointingly little about the nature of the factors involved in them. What we do know suggests that the operative factors are very different in different cases. The shape of a mass of tissue, for instance, may in one case be altered by changes in the tension in the surface of the mass, or again by changes in the adhesiveness of the cell membranes,

by differential imbibition of water, by reaction to pH gradients, by differential adhesion to other neighbouring cell masses, by the movements of its individual constituent cells, and probably in many other ways. It seems impossible to hope that we shall ever discover any single basic mechanism of pattern formation or morphogenesis, as we may still hope to find, for instance in the mechanism of protein synthesis and its control by genes, the fundamental mechanism for substantive differentiation. In discussing pattern formation and morphogenesis, therefore, one can hardly hope to do more than provide a number of illustrations of the general nature of the processes which are at work.

1. *Primary and secondary expressions of pattern*

Many of the most striking animal patterns which we can observe are probably secondary or derived expressions of the underlying primary pattern, and it is the formation of the latter rather than that of the visible configuration derived from it which presents the really interesting problem. An example will make the distinction clear. If certain hormones (thyroxin or oestrone) are injected into fowls of certain breeds a change occurs in the colour of those parts of the feathers which are being formed while the hormone content of the blood is at a high level. Lillie and Juhn (1932) studied the shape of the coloured region which is produced in response to single doses of various sizes. They showed that the threshold of hormone concentration, which has to be surpassed before a colour alteration is produced, is lowest near the rachis of the feather, and rises towards the sides. They also came to the conclusion that the various parts of the feather differ in the time-lag which has to elapse between the attainment of the hormone threshold and the actual deposition of altered pigment.

On the basis of these two variables it is easy to see that one might obtain a pattern consisting of a single spot near the rachis, by making an injection which did not raise the concentration as high as the threshold of the lateral parts of the feather. If, however, the injected dose were larger, so that it surpassed the threshold even of the lateral parts, some form of transverse bar would be obtained. The shape of this bar would depend on the relation between the time taken for the hormone level to fall again by excretion and the time-lags of the various parts of the feather. Indeed, if the hormone were excreted very rapidly the central parts of the feather, with their long time-lag, might fail to respond at all, although the concentration had for a short time reached the necessary threshold and had produced an effect on the more quickly-reacting lateral parts of the feather. This would give rise to a pattern consisting of two spots near the edges. Thus

according to the quantity of hormone injected and the rate of its excretion, quite different visible patterns might result; but they are all secondary consequences of the thresholds and time-lags which vary in a definite manner within the feather. It is the arrangement of these variations which must be regarded as the primary pattern. It is comparatively easy to understand how, once the primary pattern is given, various manifestations of it can be made visible by treatments such as hormone injections. The fundamental problem is to understand how the time-lags, thresholds, etc. come to be arranged in a pattern in the first place. (It should be mentioned that the 'classical' story of hormone-induced feather patterns, given above, has been severely criticised by 'Espinasse [1939]. It has been quoted here because it brings out very clearly the distinction between a primary pattern and the derived expressions of it.)

2. *The origination of pattern*

One of the most thorough investigations of formation of primary patterns has been concerned with the coloration of the wings of Lepidoptera (Reviews: Henke, 1935, 1948). The comparative study of nearly related species has made it possible to distinguish a number of different elements, or systems of elements, which are combined together to form the pattern of any particular wing. In the most general form there are three such systems, which in Henke's terminology are referred to as 'fields'; first, a general field, which can be thought of as covering the whole wing; second, enclosed within this is a peripheral field; and third, enclosed within that again, a central field. Both the central field and the peripheral field usually stretch right across the wing from its anterior to its posterior margin so that the general field occurs only at the base and at the lateral edge. There are usually strongly marked features at the boundaries between the fields forming a series of lines (the 'Querbinden' or transverse bands) (Fig. 20.1).

These three main fields seem to be epigenetically more or less independent. They can be very differently developed in different species. Quite often the peripheral field extends right down to the base of the wing, so that the general field disappears in this region and occurs only at the distal edge of the wing. Moreover, experiment shows that different fields are determined at different times during the development of the wing-bud. If small wounds are made by cauterisation in the developing bud after a given element in the pattern is determined, the resulting wing will show the normal pattern disturbed only by the presence of the dead area (Fig. 20.1). If, on the other hand, at the time of operation the pattern was not fully determined, its development will be modified in some way

(as we shall see later, this modification may take the form of an arrest of the pattern at an early stage in its development). If cauterisations are made at different stages it is found that some elements of the pattern may act as though they were fully determined at a time at which others are still capable of being modified. We have therefore to consider each of the fields as representing an independent unit within which pattern formation is proceeding. This means that the lepidopteran wings are rather compli-

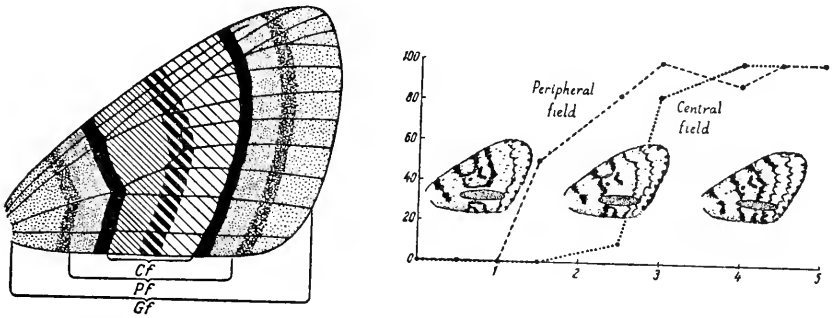


FIGURE 20.1

On left: generalised scheme for the three main elements in the wing pattern in butterflies. *Gf* indicates the maximum extent of the General Field, *Pf* of the Peripheral Field (='Umfeld'), and *Cf* of the Central Field ('Zentralfeld'). On right: the results of cauterising the pupal wing at various stages. In the graph, age at operation is given (in days after pupation) as abscissa; as ordinate is shown the percentage of cases in which the Peripheral Field or the Central Field behave in a mosaic manner which shows that they have already been determined. The three wings drawn on the graph illustrate the results of an early operation, at a time when only the spots of the edge (General Field) are determined, then a case in which the Peripheral Field is determined but the Central Field not, and finally a case in which the whole pattern is determined. (After Henke 1948.)

cated examples of pattern, since they consist of a number of different independent areas rather than a single one; but this complexity is compensated for by the great variety of patterns which are available in different species and the ease with which they can be studied.

Henke has discussed the different types of pattern which might theoretically be expected to form within any one area (Fig. 20.2). Before any pattern appears one must imagine that the area is more or less homogeneous. The simplest pattern would be a random distribution of spots of various sizes, the frequency of the different sizes falling into a normal distribution. This he calls a 'spatter' pattern. The next class he distinguishes is that in which some sign of periodicity or rhythm can be seen; for

instance, by the formation of spots of more or less similar size lying at roughly equal distances from one another, or a series of lines at approximately equal distances apart. These he speaks of as 'simultaneous rhythms', distinguishing them in this way from other rhythmic patterns which involve time, which will be mentioned later. The simultaneous rhythm may be on a small scale in relation to the whole area covered, in which

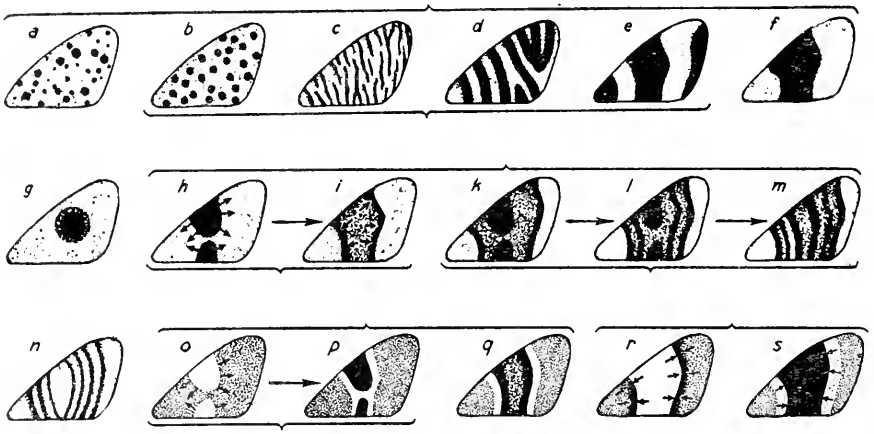


FIGURE 20.2

Types of pattern, according to Henke, illustrated in lepidopteran wings: *a*, spatter pattern; *b*, *c*, *d*, *e*, 'simultaneous rhythms' of increasing wavelength, leading to the case *f* when there is only a single element present; *g*, a single spot which has a transitional zone around it; *h*, a diffusion field; *i*, accumulation of diffusing material at the boundary; *k*, *l*, *m*, are other examples of boundary accumulations in more complex patterns; *n*, a 'centric rhythm' resulting from a Liesegang-like phenomenon in a diffusion field; *o*, *p*, *q*, successive diffusion fields; *r*, *s*, interactions of the diffusing substance with the surroundings. (After Henke 1948).

case the area will include a number of elements of the pattern. Alternatively, if the periodicity is on a larger scale, there may be few, or in an extreme case only one, repetition of the basic element.

Henke seems to imply that such rhythmic or periodic patterns can arise spontaneously within an originally uniform region. He suggests (1948) that the periodicity arises through some form of competition, presumably for diffusable substrate materials, between spots which were originally irregular in size and in disposition. A more precise account of how such regular patterns can arise has been provided by Turing (1952). We shall return to this later.

Henke then considers a number of other types of pattern which arise in

a more complicated way. For instance, each spot in a simultaneous rhythmic pattern may have not a sharp boundary but one which grades gradually into the background; or there may be a reaction between the spot and the background in such a way as to outline its periphery. Again, from a single spot or elongated area some substance may diffuse outwards and give rise to a periodic pattern as a consequence of the well-known (though little understood) Liesegang phenomenon. This form of rhythmic pattern Henke refers to as a diffusion rhythm. Mere inspection is sometimes sufficient to suggest that a given rhythmic pattern belongs to the simultaneous or the diffusion type. In the former one would expect the pattern to be rather irregular but to show no indication of any change in the wave-length, whereas in the latter one expects a more precise formation of the pattern and a gradual increase or decrease in wave-length. A final distinction of the two types can, however, only be reached experimentally.

In a number of cases, indeed, experiment has demonstrated that diffusion plays an important part in the production of a certain pattern. For instance, in the wings shown in Fig. 20.3 the central field originates from two points, one on the anterior margin of the wing and the other on the posterior. From these, two streams of some substance spread across the surface of the wing until they meet in the middle, as is shown by the fact that if the wing is wounded by cauterisation during the period when the

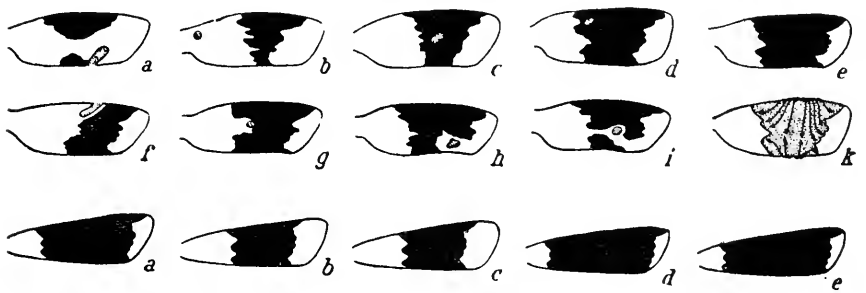


FIGURE 20.3

Diffusion as the process producing the Central Field in the wing of the mealworm *Ephestia*. The upper row show examples of inhibition of the diffusion by cauterisations performed at the time when it would normally be proceeding (2- to 3-day pupa). In the middle row, the cauterisations were made earlier; the diffusing material has merely by-passed the necrotic areas. In the lowest row, *a* shows the normal extent of the central field; *b*, a case when it is narrowed by the action of a gene *Sy*; *c*, a similar narrowing caused by high-temperature treatment during the diffusion; *d*, a widened field produced by the gene *Syb*; *e*, a similar effect caused by early high-temperature treatment. (From Henke 1935, after Kühn and v. Engelhardt.)

spread should be taking place the process is brought to a standstill, and the intermediate stages of the diffusion are revealed. Henke calls patterns formed in this way 'spreading fields'. The edges of such fields may often be outlined by some product of a reaction between the substances characteristic of the two areas.

How far does this account of some fundamental types of pattern formation, which was primarily based on the analysis of lepidopteran wings, provide us with an explanation of the kind of phenomena which we meet in the development of embryonic organs in general? It is obvious that a step of the greatest importance would have been taken if we had reached an adequate understanding of the spontaneous appearance of rhythmic or periodic patterns within an originally uniform area. Henke rather vaguely suggests that this may be due to competition between originally irregular spots. Turing (1952) in a paper with the challenging title of 'The Chemical Basis of Morphogenesis', has elaborated a mechanism by which a regular pattern might arise within a completely homogeneous system. He considers a region (imagine a plane two-dimensional area to make it simpler) in which a number of chemical reactions are proceeding. If these interact with one another by involving the same substances, or by producing products which act as catalysts or affect the rates of other reactions in any way, then the straightforward situation would be the attainment of some sort of balanced equilibrium condition throughout the whole area. But such an equilibrium is only a statistical phenomenon; actually the system will be disturbed by slight chance variations from place to place. Now it is easy to imagine special systems of reactions such that the equilibrium is unstable; if by chance one substance appears at a certain place in slightly too high a concentration, it will go on increasing. From each such 'high' spot, the substance will diffuse outwards, so that the spots will gradually enlarge. Turing has set up mathematical equations for such systems, and, choosing some arbitrary figures to express the rates of the reactions and of the diffusions, has solved them by means of a modern computing machine. He found that under certain conditions one might expect to get a pattern of a few fairly large areas or irregular blotches of high values of some particular substance. Moreover in some circumstances, the pattern might be more regular, showing a rhythm with a definite wave-length dependent on the physical and chemical magnitudes controlling the reactions.

Turing compares his 'chemical wave-length' with the interval between regularly appearing structures in an animal or plant. For instance, if the circumference of the cylindrical body of a *Hydra* were just about six times the wave-length, one might attribute the animal's hexagonal symmetry,

and the appearance of six tentacles, to such a mechanism. But this does not seem very convincing. One of the most important characteristics of embryonic development is that the patterns which arise tend to be accommodated to the total amount of material available. For instance, a normal embryo can be formed from half an egg, or from two eggs fused together; or if a flatworm is cut longitudinally into strips each much narrower than the normal breadth of the worm, nevertheless each of these will regenerate a complete head. In patterns which behave in this way, the distance apart of the various elements cannot be fixed by any definite chemical wave-length dependent on the unchanging values of rate constants, diffusion constants, etc. Rather the pattern must arise as a whole within the boundaries of the material available.

The simultaneous rhythms of Henke or the chemical rhythms of Turing cannot, then, provide a general explanation of the periodic patterns which are important to animal morphology. It seems in any case improbable that fundamental rhythmic patterns, such as those of the somites of the vertebrate body, would be dependent on such an inherently chancy mechanism as that investigated by Turing. Probably the processes which he and Henke have discussed play a part only in the quasi-periodic dapplings and mottlings which often fill up relatively unimportant spaces.

The fact that a developmental pattern is usually found to become either enlarged or diminished in scale so as to fit into the available material suggests that the boundaries of the mass of substance play a major part in the processes by which the pattern is formed. For instance, in a regeneration blastema of a flatworm, it may be that some process always attains a critical value in the midline and falls off to zero at the two lateral edges. If this, or something like it, were the case, one could understand how a complete head appears even on a very narrow strip. Again, in Henke's wings which are characterised by a central field, we may suppose that the position on the anterior margin from which the diffusion of the field starts is always midway between the base and the tip, however large or small the wing may be. It is only by some such relations as this, in which the pattern is produced in dependence on the boundaries of the material available, that the facts as they are observed can be adequately understood.

Although, as has been stated above, developmental patterns often retain their completeness even when the material available is considerably greater or less than normal, this is not always the case. The relation between pattern and mass is certainly not simple and probably differs in different cases. A number of other instances will be mentioned below.

3. *Some actual patterns*

It is now time to turn from this somewhat abstract discussion of the fundamental principles of pattern formation to the consideration of one or two actual examples of patterns which have been relatively fully analysed. Studies employing essentially biological methods (e.g. investigation of the development of mutant types, or the performance of surgical operations) have shown that many apparently simple patterns result from the interplay of numerous factors. Investigations by chemical or physical methods have not as yet progressed nearly so far. Indeed in most cases we have no actual evidence at all as to the physico-chemical nature of the processes involved and can hardly proceed beyond such *a priori* arguments as those discussed above.

(*a*) Drosophila *wing venation*

It is, as might be expected, in *Drosophila* that genetic methods have provided us with the greatest mass of information concerning patterns. Most of the important principles which emerge from such studies can be illustrated by a consideration of the venation of the wings (Waddington 1940*b*). The veins of the adult wing present a fairly simple pattern, consisting of five longitudinal veins running from base to tip, with two cross-veins, an anterior one between *L*3 and *L*4 and a posterior between *L*4 and *L*5. This pattern arises in a series of stages, of which we may distinguish three: (1) The prepupal stage, in which *L*2 is absent, *L*3 and *L*4 are united from the base to near the middle of the wing blade, there is a marginal sinus right round the edge (part of which corresponds to the later *L*1) and there are no cross-veins. At the end of this stage the wing is inflated into a balloon-like shape by the pressure of the internal fluid and all visible traces of the prepupal venations disappear. (2) When the wing contracts again, in the pupal period, the five longitudinal veins make their appearance. (3) In a slightly later phase, at the very end of the contraction, the two cross-veins appear (Fig. 15.1, p. 331).

Some genes have effects which affect the five-rayed pattern of the longitudinal veins as a single organised unit (Fig. 20.4). The effects of these generally acting genes are, however, of several different kinds. Perhaps the most striking, but the least illuminating with regard to pattern formation, are genes such as *dumpy* which, by affecting the pupal contraction, distort the pattern of veins after it has been laid down. This is not really an effect on pattern formation, but only on the expression of the pattern. Some other effects, however, are more radical. For instance, in *shifted* all the veins from *L*2 to *L*5 appear as though squeezed together, diverging at a lesser angle. This occurs without any noticeable change in the outline

of the wing; the effect on the pattern formation must be brought about by altering the reaction of the material to effects of the wing boundary, rather than by altering the boundary itself. Other cases, however, suggest that the amount and shape of the material available to form the wing may have an effect on the pattern of venation. For instance, in *dachs* the longitudinal veins are splayed apart and at the same time the wing is

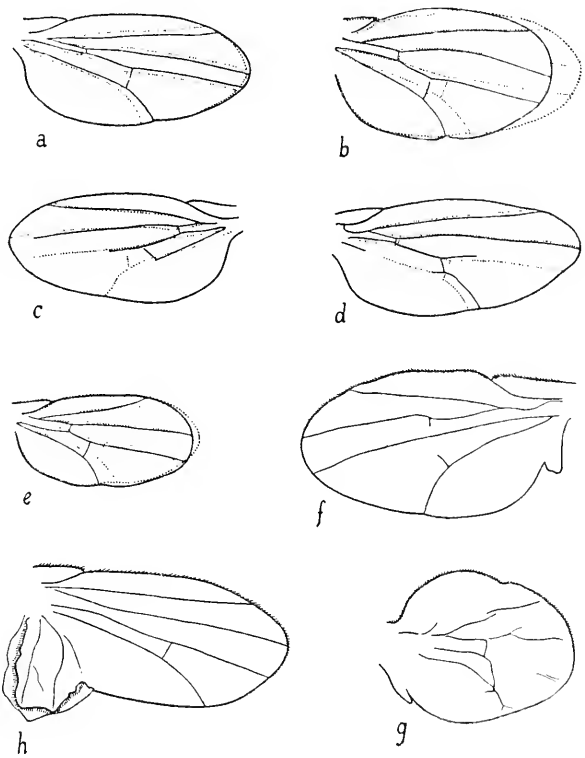


FIGURE 20.4

Gene-controlled modifications of the pattern of wing venation in *Drosophila*. In *a*, *b*, *c*, *d*, *e* the mutant wing is drawn in a full line, superposed on a dotted drawing of a normal wing; *a*, in *shifted-2* the longitudinal veins are pinched together; *b*, in *broad*, the wing blade is relatively broader than normal and the veins diverge at a greater angle; *c*, *veinlet* may remove a considerable part of the distal region of the veins, and the posterior cross-vein is moved to fit; *d*, *cubitus interruptus* removes the distal part of the fourth vein, and the fifth then shifts upwards; *e*, *dachs* produces a short square wing, in which the veins diverge at a greater angle than normal; *f*, the *dachsous* wing is large and the veins diverge at a large angle, notice also the cross-veins; *g*, much-altered venation when the wing shape is highly abnormal (*dachsous-fourjointed-plexus*); *h*, small mirror-image twin wing produced by *blot*. (After Waddington 1940, etc.)

square in shape, and it seems probable that it is the change in wing shape which has brought about the alteration in the laying down of the pattern.

It is not quite clear what happens to the pattern if the size of the wing is reduced, without alteration of its shape, before the venation is determined; genes which produce small wings (such as *miniature*) act largely, if not wholly, after the pattern has been laid down and thus, like *dumpy*, distort something which is already in existence rather than alter the conditions under which it comes into being. Genes are known, however, which cause increases in the wing mass earlier than the period of pattern formation. One is *dachsous*; this usually produces a fairly slight increase in size and a five-rayed pattern of longitudinal veins appears, the angle of divergence being, of course, larger than usual. In *blot*, on the other hand, the exaggeration in size is greater, and extra longitudinal veins make their appearance. In extreme cases these extra veins can be seen to form a mirror-image of the normal venation, the mirror plane being along the position of *L*5. Such duplication and mirror-imaging is rather common when a pattern is developing in a mass of tissue which is, as it were, too large for it. One feels that it should offer an important clue as to the nature of the essential processes concerned in pattern formation, but so far no one has suggested just how it should be interpreted: stimulating discussions are given by Harrison (1945), Needham (1936*a*).

The whole set of five longitudinal veins does not, however, always behave as a unit. There are certain genes which have localised effects on particular veins or particular parts of veins. House (1952) has been able to show that some genes which appear to have strictly localised effects may exert on neighbouring regions sub-threshold influences which are not strong enough to produce any actual alteration except in combination with other genes; but even if this is the case, it remains true that these genes affect most strongly particular sections of the venation rather than the system as a whole. One gene of this kind which acts at an early stage is *cubitus interruptus*. This causes an absence of the distal end of *L*4 in the prepupal stage, and this vein does not reappear and is also missing in the adult. It is interesting to find that in the adult wing the tip of *L*5 moves forwards, as though to try to fill the space left by the absence of *L*4. We have, then, a certain reaction of the pattern as a whole to the local defect which is the primary effect of the gene. This reaction of the whole system probably occurs at the second phase, that of the pupal contraction, the absence of *L*4 having been produced earlier, in the prepupal phase. Most local absences of veins, such as those caused by *veinlet*, *tilt* or *radius incompletus*, do not occur until the second phase, and in these cases there

is no sign of any general reaction of the pattern to compensate for the local absence.

The last-formed element in the pattern—the posterior cross-vein—always adjusts its position in relation to any previous event which has affected its general neighbourhood. For instance, if the longitudinal veins are splayed apart at their first inception, e.g. by *dachs*, or even by differential growth immediately after their formation, e.g. in the mutant *broad*, then the posterior cross-vein moves inwards towards the base of the wing. It reacts to absences of the distal parts of the longitudinal veins *L*4 and *L*5 even when these are produced by genes acting as late as the pupal contraction, such as *veinlet*. It can also be moved by surgical operations made at a similar time (Lees 1941). These variations in the position in which the posterior cross-vein appears make it very obvious that the pattern is an expression of an equilibrium resulting from the interaction of numerous factors.

Two other observations on wing venation may be mentioned, to emphasise the complexity of the underlying epigenetic processes and the equilibrium character of the simple pattern which is normally produced. Timofeeff-Ressovsky (1931) studied a gene *Vti* (*venae transversae incompletae*) in *Drosophila funebris*. This causes a break in the posterior cross-vein. He found it possible, by selection, to isolate different stocks, in one of which the break occurred at the anterior end of the vein, in another at the posterior end and in the third at both ends with equal frequency. The genetic differences between these stocks depended on numerous factors of small effect. Since each of the genes concerned is presumably producing its own specific effect, we must conclude that very many individual processes are involved in this very detailed determination of a small part of the venation pattern. Again, another gene in the same species, also studied by Timofeeff-Rissovsky (1934), produces an effect which can be considered as a general disturbance of the condition of equilibrium of the vein-forming processes; the effect is either that many parts of the vein are missing or that large amounts of extra vein material are formed.

The thoroughness with which the venation pattern can be analysed, thanks to the large number of mutant forms available, has revealed a number of general points which are probably applicable to other cases of animal pattern about which we have as yet less actual knowledge. In the first place we see that even a comparatively simple pattern, such as that of the *Drosophila* wing venation, may be composed of a number of different parts which arise relatively independently and in this case at somewhat different times. Thus the main longitudinal veins *L*3, *L*4 and *L*5, pass

through two phases in the prepupal and pupal stages, while *L2* and the posterior cross-vein belong to a different system, since they are not represented in the prepupal wing; and for the posterior cross-vein in particular we have clear evidence that it is determined at a later stage than the longitudinal veins. Each system within the pattern must have a complex epigenetic basis, since genes exist which can alter it in a number of different ways. Sometimes the system reacts as a whole, as in *dachs* (where the shape of the wing is important) or in *shifted* (where it is not); but different parts of the system may have their own characteristic properties, exhibited for instance in the localised effects of *cubitus interruptus* or *tilt*. If a part of the pattern is removed at an early stage in the developmental processes, compensatory phenomena may occur later. Finally we may note that when the initial mass of material is much larger than normal, as in *blot*, there is a tendency for the whole pattern to be duplicated, the duplicate being a mirror image of the normal.

All these facts force one to conclude that a pattern represents a gradually developing equilibrium between a number of forces. The system of forces may be very complex, and include a large number of different items. This might suggest that it is almost hopeless to try to obtain any fuller understanding of the genesis of a pattern. However, although the total number of forces involved may be unmanageably large, it is quite probable that only a few of them play major roles. For instance, the wing veins represent cavities which persist when the originally hollow sack of the wing contracts and presses out the body fluid with which it was originally filled. It is clear that the tension in the wing epithelia, and the hydrostatic pressure of the body fluid, must be among the important factors with general effects on the process. T e other main element in the situation is the set of factors which determine that there shall be only four (and not perhaps five) longitudinal veins between the anterior and posterior margins of the wing. It might not seem too optimistic to hope that we could discover the nature of the processes which determine this major feature of the pattern. It must be admitted, however, that as yet we have scarcely any clue even as to the general type of phenomena which comes into question. Are we dealing with diffusion, with the elastic and viscous properties of the membranes, or with—what?

(*b*) *The pentadactyl limb*

As another example of an embryonic pattern we may briefly consider the appearance of the bony structures in the vertebrate limb. In the normal hindlimb there is a single femur attached to the tibia and fibula, at the end of which are the five digits. This fundamental pattern first appears as

condensations within the mass of loose mesenchyme which makes up the body of the limb-bud (Fig. 20.5).

The first point we may notice is that the pattern is not at all closely dependent on the mass of material available. If a limb-bud is halved at an early stage, the complete pattern may appear within the half-sized part. There is indeed a considerable tendency in limb-buds which have been

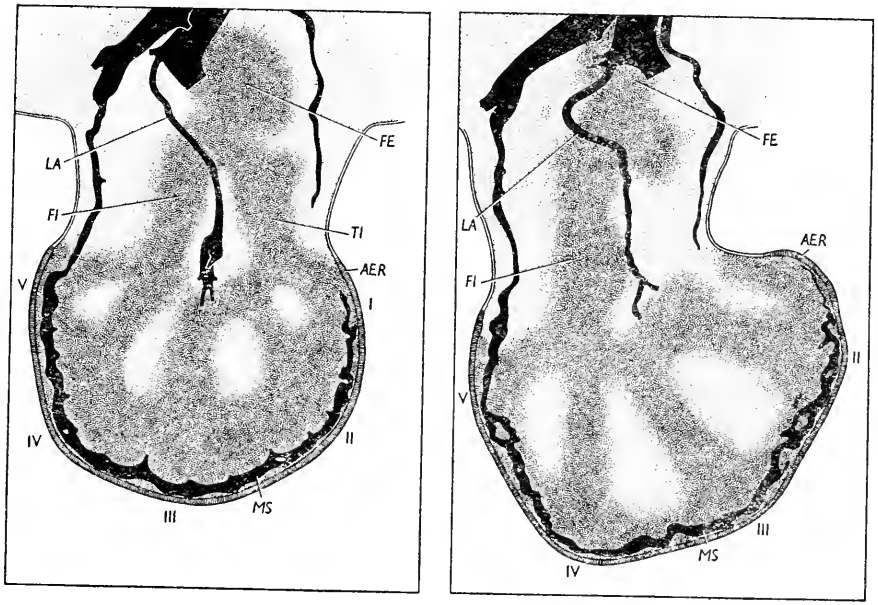


FIGURE 20.5

On the left, the structures in the right hindlimb-bud of a normal 12½-day mouse embryo, projected on to the plane of the footplate. The mesenchymal thickenings for the femur (*FE*), fibula (*FI*), tibia (*TI*) and digits are visible. *AER* is the apical ectodermal ridge. On the right is the disturbed pattern found in a *luxate* homozygote. The pre-axial side of the limb-bud is enlarged, but the blastema of the tibia is absent. (From Carter 1954.)

disturbed in some way, for instance by transplantation to other sites, for a spontaneous subdivision to occur, so that duplicate limbs are formed. These are nearly always mirror-images of one another. If the division takes place at a somewhat later stage and is incomplete, partial duplication may occur, giving rise to structures with more than the normal number of toes.

Such polydactylous limbs are also produced by a number of genetic factors. In some of the extreme forms, particularly in birds (Reviewed: Waddington 1952*a*, Landauer 1948) the genetically caused polydactyly

may also be fundamentally a duplication. In other cases, however, the condition represents an alteration to the basic pattern by the addition of elements to it, rather than a duplication of a pattern which remains essentially unchanged. For instance, in the guinea-pig the forefoot normally has four digits, and the hindfoot three. Wright (1935) has described a dominant gene which when heterozygous produces some tendency towards the formation of extra digits. By selection Wright built up a race in which the general genetic background was such that the heterozygote rather regularly had five toes on the forefeet and four on the hindfeet. There is no evidence that this represented a partial duplication of the normal pattern; it seems rather to be a straightforward modification of it. Animals homozygous for the gene had feet with very large numbers of toes which again showed no evidence of representing multiplications of the basic four- or three-toed patterns. It is noteworthy, as an indication of the complexity of the reactions whose equilibrium is represented by the normal pattern, that in order to obtain the regular addition of a single toe it was necessary not merely to have a single dose of the main gene, but also to select a large number of other appropriate genes in the genetic background.

The development of low-grade polydactylous limbs in mammals has recently been carefully studied by Carter (1954) in the luxate strain of mice. Polydactyls occur both among the heterozygotes and the homozygotes for this gene (Fig. 20.6). The first effect noticeable during their development is an overgrowth of the anterior (pre-axial) side of the limb-bud. In this region the condensations of mesenchyme are irregular, and extra condensed regions may appear from which the supernumerary

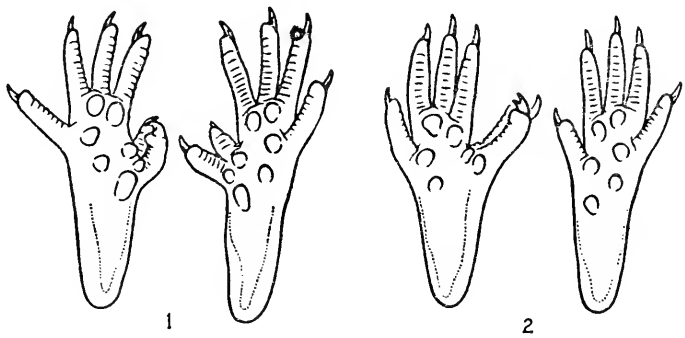


FIGURE 20.6

The hindfeet of two *luxate* heterozygote mice, illustrating various grades of polydactyly. The left foot of the second animal (on the right of the drawing) is normal. (From Carter 1954.)

digits will be produced. It is a remarkable fact that in the homozygotes, which of course show the more extreme expressions of the gene, skeletal elements are frequently missing (not only pre-axial digits but also the tibia). Thus the same genetic influence, which at fairly low intensity produces polydactyly, in higher intensity has an effect of a rather opposite character. Carter tentatively suggests that a field responsible for producing the five-toed pattern, and perhaps inherent in the apical ectoderm (see p. 276) is in the luxate heterozygotes shifted anteriorly in relation to the underlying competent mesenchyme. This draws into the process of limb formation material which would normally lie beyond the range of the limb-inducing influence, and this may account for the increased size of the pre-axial region. To explain the reduction in the limb skeleton in the extreme cases he supposes that the pattern-inducting field is shifted so far anteriorly that part of it overlies mesenchyme which is not competent to respond.

This hypothesis provides a formal explanation of the facts, but still leaves one quite in the dark as to the nature of the processes which cause the limb mesenchyme to form a certain number of condensations. It is probably significant that in polydactylous limbs it is normally on the pre-axial side that the extra digits appear. Gabriel (1946) found that if the opposite (post-axial) side of the limb-bud of a polydactylous strain of chicks was inhibited in growth by treatment with colchicine the polydactyly was exaggerated. This suggests that one aspect at least of the normal process of pattern formation involves a control of the pre-axial side by the post-axial, this control being weakened when the post-axial side is inhibited. It is perhaps simplest to imagine the control involving the diffusion of substances, but there is still no direct evidence of this. Indeed, once again one has to admit that we have no clear-cut indication even of the general category of process to which we ought to look to find an explanation of the apparently simple fact that an originally homogeneous mass of mesenchyme begins to draw itself together into a number of separate regions of condensation. Phenomena of this kind obviously lie at the basis of the whole of animal morphology and our almost total ignorance as to how they are brought about offers a challenge which it is to be hoped experimentalists will soon take up successfully.

In the last few paragraphs we have considered the original initiation of the pattern of the limb skeleton. It must of course be remembered that this may undergo modifications of quite a fundamental character after its first formation. For instance, Tschumi (1953) showed that if the young limb-buds of the toad *Xenopus* are treated with colchicine there is a considerable inhibition of growth which leads to a reduction in the size

of the toes. The different toes do not react equally, and although they fall roughly into order from more sensitive to less sensitive, the effects are somewhat irregular as between different animals Tschumi found that if a toe were reduced below a certain minimal size at the time of its first appearance it did not persist in later development but eventually disappeared, probably owing to a process of the nature of competitive interaction, by which the other toes drew away from it all the available supplies of essential metabolites (cf. Lehmann 1953). Thus four-toed or three-toed conditions may arise secondarily, if there is a sufficient disparity in size in the digits as they are originally formed.

One cannot close even such a short discussion of animal pattern as this without referring to the famous work of D'Arcy Thompson, *On Growth and Form* (1916). By showing that many animal forms share certain mathematical properties with shapes that are known to arise in the inorganic world, D'Arcy Thompson had a most important influence, both in persuading biologists that form offers a problem which should be analysed in causal terms, and in making it seem not too impossibly difficult for such an analysis to be carried out. These services were very great ones; but nevertheless nearly all this task of understanding remains for the future. A large part of D'Arcy Thompson's work dealt with one special category of forms, namely those of simple cells and of small groups of cells. Even if one accepted his discussion, which is framed in terms of the surface tension of liquid films—and would nowadays be rejected by many who feel that the cell membrane cannot be regarded as a liquid—still one would be forced to admit that the principles he discussed throw little light on the initiation of a pattern such as that of a pentadactyl limb. Again, he discussed the forms that arise from particular types of differential growth, such as that which causes the shell of a gastropod or a cephalopod to be twisted into a spiral; but interesting though that is, it leaves unsolved the fundamental question of how the pattern of differential growth rates arises in the first place. The most essential problem of form is one which cannot be approached by a mathematical analysis of the ways in which animal shapes become transformed during development. It is the question of how form originates from the formless, and demands either an experimental attack or a mathematical analysis of a different kind, perhaps similar to that begun by Turing.

Perhaps the most cheering thing that can be said about the problem of form is that it does not, perhaps, pose itself in its full intensity as often as might appear at first sight. The majority of animal forms develop out of something which already has some degree of pattern within it. For instance, an egg, as we have frequently seen, is by no means the com-

pletely uniform and homogeneous body which it seems, but has certain elements of structure which provide a basis on which the more elaborate patterns of later development may be constructed. Even in examples such as those we have chosen to discuss, of the *Drosophila* wing and the vertebrate limb-bud, where the earliest stages show no obvious sign of even a trace of inherent pattern, it may well be that this apparent homogeneity is partly deceptive. Perhaps after all we are never confronted with the origin of pattern from the completely formless but only with increases of complexity of pattern. But even so, the problem of how this occurs is difficult enough and at present almost completely beyond our understanding.

4. *Morphogenesis*

The word 'morphogenesis' is often used in a broad sense to refer to many aspects of development, but when used strictly it should mean the moulding of cells and tissues into definite shapes. Morphogenesis, like pattern formation, is thus a phenomenon which is involved in almost every instance of differentiation. It is clearly impossible to discuss everything that is known about it. We shall therefore consider only one or two examples which will serve to illustrate the nature, and the successes and limitations, of some of the approaches which have been made to the problem.

(*a*) *Movements of isolated cells*

One of the simplest types of morphogenetic process is oriented movement by isolated cells. Weiss (1933, 1945) has devoted particular attention to this as a factor in normal development. By experiments on cells growing in tissue culture, he has shown very clearly that when cells creep about by any form of amoeboid movement a very powerful influence on the direction of movement is exerted by the microstructure of the medium or surface on which the cells are placed. Thus if the cells are provided with a fibrous substratum, such as glass wool, they tend to creep along the fibres. The same is true when the fibres are sub-microscopic in scale. If a plasma clot is stretched, the protein micelles of which it is composed become partially oriented, and both the shape and the direction of movement of the cells fits into the ultra-structure of the medium (Fig. 20.7).

Weiss points out that in a developing embryo the intercellular spaces are filled with a 'ground substance' or protein-containing jelly. The differential growth of different parts will stretch this in certain ways, and by thus orientating the ultra-structure of the ground substance, influence

the direction of migration of any mobile cells present. Such processes almost certainly play a very important part in events such as the outgrowth of nerve fibres and the migration of the neural crest cells in vertebrate embryos. They may also be involved in some of the morphogenetic behaviour of rather more closely packed aggregations of cells. They are one of the mechanisms one might look to in attempting to understand the origin of the five-rayed pattern in the limb-bud, which was discussed above without reaching any conclusion as to the fundamental mechanism involved. On the other hand, this mechanism can scarcely be held to account for the movements and behaviour of tissues such as those involved in the gastrulation of the Amphibia or the rolling up of the neural plate.

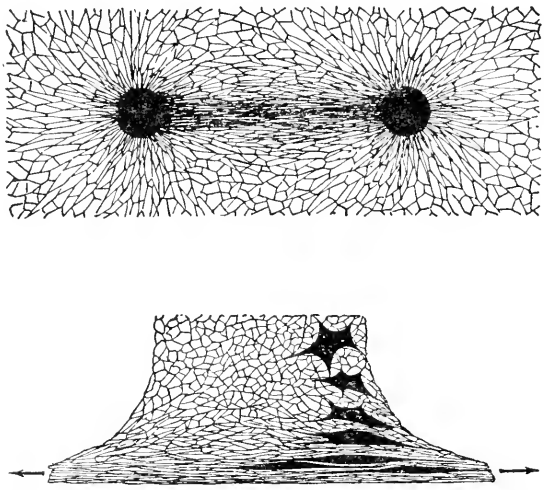


FIGURE 20.7

Above, diagram of the orientation of a reticular matrix between two centres of contraction. Below, diagram of the effect of stretching (between the arrows) on the shape of mesenchymal cells growing in a reticular matrix. (After Weiss 1949.)

Another investigation on the movement of isolated cells which may have a considerable bearing on morphogenetic processes in general is centred around the rather peculiar situation in the amoeboid slime moulds (*Acrasiales*). During one stage in their life-history these organisms exist as isolated amoeboid cells. If these are cultured on a surface of nutritive agar, they at first move about in an uncoordinated and haphazard way. Growth and cell division continues until the density has reached some

critical value, when a new process starts. This is aggregation. The amoebae move together into streams which converge on a certain region, the aggregation centre, at which they become heaped together to form a sausage-shaped cell mass (Fig. 20.8). This mass then starts moving as a whole, creeping over the surface at a speed comparable to that of the individual amoebae (approximately 2 mm. per hour). After a time the

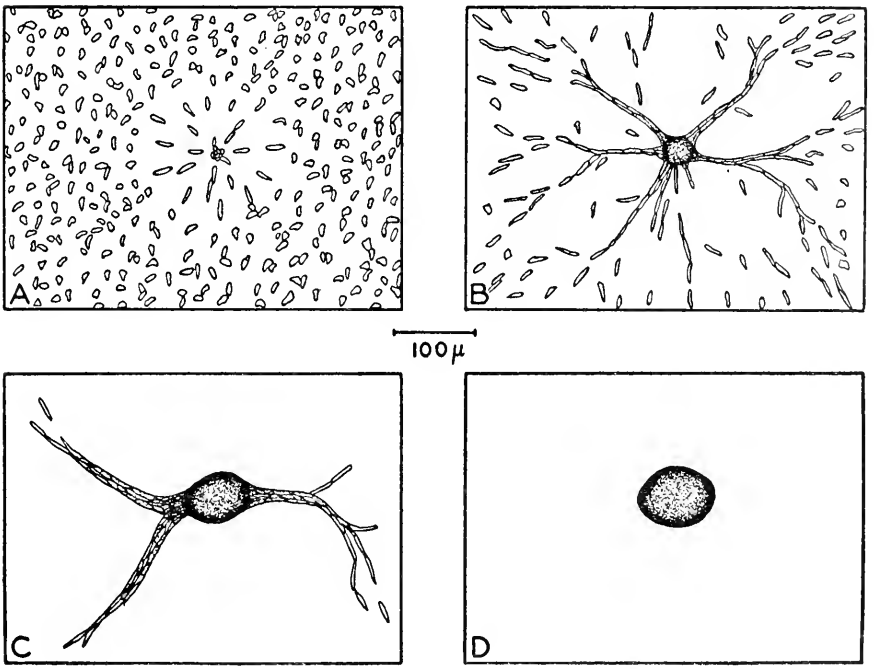


FIGURE 20.8
Four stages in the aggregation of *Dictyostelium* from the amoeboid phase to a compact mass. (From Bonner 1952.)

creeping movement stops, and the heap gradually rears itself up into a peg-like structure, with a lump of cells (which become spores) at the top of a thin stalk which stands on a small expanded base (Fig. 20.9). Now it has been shown (particularly by Bonner 1947, 1952) that the first phase of these morphogenetic movements, namely the aggregation, occurs under the influence of a substance, known as acrasin, which is given off by the amoebae. Each amoeba gives off this substance, and at the same time tends to move along any gradient of acrasin that may be present. If sufficient amoebae happen to close together they form a

centre of high acrasin production, and other amoebae will move towards them.

The behaviour of the amoeboid stage of the slime mould is important in providing a clear-cut demonstration that cells can attract their like from a distance. It seems quite probable that processes of this kind play a part, along with the orientation of the ground substance, in controlling the migrations of isolated cells during embryonic development. One must remember, however, that even in the slime moulds the existence of external gradients of acrasin concentration is not sufficient to explain the whole range of the phenomena we observe. For instance, the movement of the sausage-shaped lumps of aggregated cells do not seem to be directly dependent on external acrasin gradients. In these movements, and in the processes leading to the formation of the peg-like fruiting body, Bonner

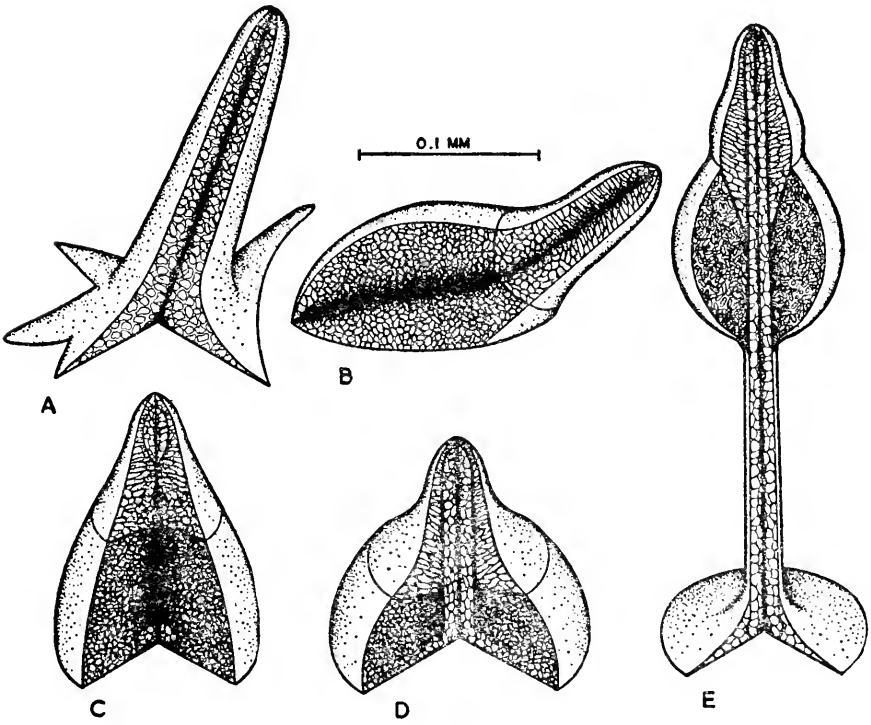


FIGURE 20.9

Stages in the formation of a fruiting body from the aggregated mass of *Dictyostelium* cells. *A* is during the migration of the mass. In *B* and *C* the mass is settling down, in *D* and *E* the cells at the tip of the mass are becoming elongated stalk cells which push down the axis to the substratum and then raise the whole mass into a peg-like structure. (From Bonner 1952.)

suggests that influences arising from the contact and adhesion between the walls of the individual cells must be involved in addition to the acrasin gradients.

It is worth pointing out that the whole sequence of aggregation, movement of the aggregated mass, and eventual formation of the fruiting body, exhibits, as all organic development does, the formation of a definite pattern of differentiated organs as well as mere movement.

Twitty (1949, Twitty and Niu 1954, Flickinger 1952) has devoted considerable attention to the factors controlling the migration of the pigment-forming cells from the neural crest in Amphibia. He has shown that one of the main factors is a tendency for the cells to move away from each other. This tendency is increased when the cells are in a closely confined space, which makes it probable that the underlying cause is a movement away from concentrations of waste products. This is the reverse of what we see in the slime moulds, in which in the aggregation phase the amoebae tend to move together. However, the pigment cells in different species of newt exhibit rather different properties in this respect. Whereas those of *Triturus rivularis* merely tend to disperse and to remain dispersed, those of *T. torosus*, after first dispersing, then tend to move back together again into clumps. The location in which these clumps form is influenced by the underlying mesodermal structures, and again there are differences between the species in these mesodermal influences. For instance, some factor in the *torosus* mesoderm prevents the migration of the pigment cells beyond the dorsal margin of the yolk mass, but this impediment is not offered by the *rivularis* mesoderm. Its nature is still unknown. Twitty claims that there is little evidence that oriented fibrillar structures in the ground substance, such as those postulated by Weiss, play any part in controlling these pigment cell migrations.

Abercrombie and Heayman (1952, 1953) have also studied the movements of isolated cells, in this case chick fibroblasts in tissue culture, and have emphasised the fact that contact between cells often brings their migration to a halt, these cells apparently having a strong tendency not to creep over one another.

(*b*) *Movements of tissues: amphibian gastrulation*

Probably the most fully studied instance of morphogenesis and pattern formation by a tissue is the gastrulation and development of the embryonic axis in Amphibia. The morphogenetic aspect of this comprises the tissue movements of gastrulation, the rolling up of the neural plate into a neural groove and tube, and the subdivision of the sheet of mesoderm into a notochord with rows of somites on each side of it. The pattern formation

involves, firstly, the determination of the plane of bilateral symmetry of the egg, and then the appearance of regional differences within the organiser (head organisers, tail organisers, etc.) and of a dorso-lateral field within the mesoderm. Clearly the pattern formation and the morphogenesis are inextricably involved with each other, and can only be separated conceptually by roughly classifying some of the events as rather more chemical in nature (and therefore related to pattern formation) and others as more definitely physical (and therefore connected with morphogenesis). For convenience of discussion we shall start by considering some of the morphogenetic processes which various authors have postulated, and shall then consider the nature and development of the patterns (p. 455).

During gastrulation we are confronted with massive streaming movements by which the tissues are moved from one place to another. One hypothesis about the causation of such movement would be to suppose that certain regions are undergoing more rapid growth than others and that the tissue streams are due to the expansion of the growing regions. However, careful studies, particularly by Pasteels (1942*b*), have shown that in gastrulating embryos mitosis goes on at a more or less uniform rate in all regions. Similarly Gillette (1944) has shown that differential mitosis cannot be held responsible for the rolling up of the neural plate into the neural tube in Amphibia. Thus differential growth, important though it may be at later stages, is certainly not a major factor in the morphogenesis of the gastrula-neurula stages.

Again it might be suggested that a differential expansion of the cells in certain regions, caused by the imbibition of water, might be the underlying cause of the morphogenetic movements. It has been found, however, that there is very little change in specific gravity of the cells during neurulation, and there can thus be little inhibition of water (Brown, Hamburger and Schmidt, 1941), so this hypothesis also is inadequate.

Another type of process, which could be postulated to account for changes in cell shape similar to those which might be produced by differential absorption of water, is the formation of fibrous structures in the internal cytoplasm. One might expect that if the cytoplasm becomes fibrous in character, the fibres would tend to lie parallel to one another and give rise to an elongation of the cell, or parts of the cell. Such parallel orientation of fibres could, in favourable circumstances, be detected by polarised light, since it should cause some degree of double refraction in directions dependent on the sub-microscopic orientation. This undoubtedly occurs in the mitotic spindle; and membranes, such as the nuclear

membrane and the external cytoplasmic membrane, often show a double refraction due to the orientation of the molecules composing them (Reviewed: Frey-Wyssling 1948).

In most cells, however, there is little evidence that the bulk of the internal cytoplasm also contains orientated structures of this kind, although during many of the early morphogenetic processes in amphibian embryos some cells assume elongated wedge-like or flask-like shapes, such as might be expected on this hypothesis. This occurs, for instance, in the cells lining the early blastopore, in the neural groove and in ectodermal in-foldings such as the lens. Polarised light cannot be used in these cases, since the cells are still full of yolk granules which are highly refractile and obscure the picture presented by the cytoplasm itself. However, the yolk granules are not spherical, but somewhat ovoid, and if the cytoplasm possessed any strongly oriented fibrillar structure, one might expect that the yolk granules would lie with their long axes parallel to it. Inspection of sectioned material does not reveal any clear evidence of such orientation (Waddington 1942*c*), and it is therefore probable that intracellular fibres play at best a very minor role in amphibian early morphogenesis (at least, directly, cf. Lawrence *et al.* 1944). The nuclei, which are of course much larger than the yolk granules, are usually very definitely orientated in the same direction as the main body of the cells, and the appearances strongly suggest that this is because they have been squeezed into these positions by the constraining cell walls, which would therefore appear to exert an important effect in determining the shape of the whole cell.

Recent work on the forces producing morphogenetic change in the amphibian embryo has, in fact, been led from several points of view to attach great importance to the behaviour of cell membranes, both those between the cell and the external medium, and between cell and cell. As has been particularly emphasised by Holtfreter (1943*a*, 1943–44), the external (cell-medium) membrane of the early amphibian egg has several peculiar properties. Its outer surface is more or less solid and non-adhesive, and it has a great capacity both for elastic expansion and contraction and for plastic flow. As the egg becomes segmented this surface layer keeps fusing up again across the newly appearing cleavage furrows, so that it remains as a continuous undivided sheet (the so-called 'coat') connecting the cleavage cells. It is, indeed, the main thing which holds the cells together. If it is dissolved by treatment with alkaline solutions the cells fall apart, since there is little tendency for the internal cell membranes to adhere to one another at this stage. As well as binding the cells together, the coat has also important osmotic properties, being less permeable to

most substances than are the membranes bounding the inner surfaces of the cells (Fig. 20.10).

There seems little doubt that expansions and contractions of the coat are important factors in early morphogenesis. Holtfreter pointed out that if unfertilised eggs are kept in media of different osmotic pressure for several days, the pigmented coat of the animal half tends to expand in a manner which reminds one of the expansion of the animal pole region of the blastula during gastrulation. Indeed, after treatment with Ringer solution, the expanded animal coat may dive into the interior of the egg in a way which quite closely simulates the process of invagination. This suggests that the coat has some inherent capacity to carry out such

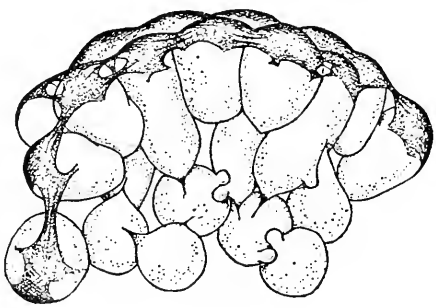


FIGURE 20.10

Fragment of a morula of the axolotl, showing the pigmented surface coat. (From Holtfreter 1943.)

movements, independently of the cellularisation of the underlying material. It would be interesting to know whether the expansion of the coat is connected with the formation of any oriented fibrous micro-structure within it. The fact that the expansion is greater in the one plane, in which the 'invagination' takes place, suggests that this is the case, but there has been no direct demonstration of it owing to the difficulty of polarised light studies on such material.

In stages later than gastrulation the coat may also have important morphogenetic effects. If small groups of cell are placed in an air/liquid or liquid/liquid interface, the cell membranes will be disrupted if the surface tension is powerful enough. By using a number of interfaces of appropriate surface tensions, Waddington (1942*c*) showed that the effective strength of the coated surfaces of cells from gastrula and neurula stages gradually increases as development proceeds, and that as the neural plate

rolls up into the neural groove the strength of the outer concave surface becomes markedly greater than that of the inner convex surface. Such an increase in the strength of the concave surface would be produced if the coat there was undergoing contraction and thickening, and it seems rather probable that this is occurring and is one of the major factors in causing the change of the neural plate into the neural groove (Fig. 20.11).

The whole process of gastrulation cannot, however, be attributed to changes in the coat. Amphibian gastrulation involves two main types of movement for which an explanation has to be found. If one compares the shape of the presumptive areas in the late blastula with the configurations which they will have assumed at the end of gastrulation, the major changes would be accounted for if we suppose that an area lying in the

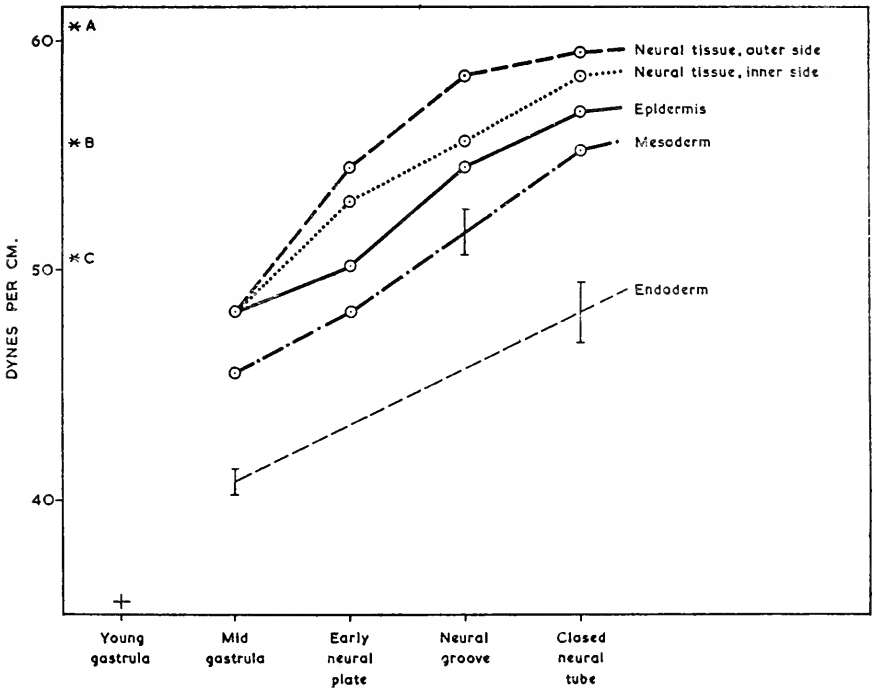


FIGURE 20.11

The ultimate strength of various tissues in amphibian embryos. The figure for the blastocoel roof of the young gastrula (indicated by a cross) was found by pulling a small steel ball through the tissue with a magnet. The other figures were derived from observations of the rate at which the cell surfaces were disrupted when placed in the air-liquid interface of the three saponin solutions whose surface tensions are given at *A*, *B* and *C*. The measurements are approximate, but indicate the changes which occur, and the relative strengths of the surfaces. (From data of Waddington 1939*b*, 1942*c*.)

dorsal midline became longer from anterior to posterior and at the same time narrower from side to side, while in more lateral regions these two changes in dimensions were less marked. Indeed, on the ventral side there would have to be some increase in the side-to-side dimension to compensate for the narrowing that occurs near the dorsal plane. This locally-variable change in dimensions constitutes the first factor to be accounted for. The second is the fact that the dorsal material, as it increases its anterior-posterior length, is tucked inside the blastocoele to form a primitive gut instead of merely protruding as a process sticking out from the region of the blastopore.

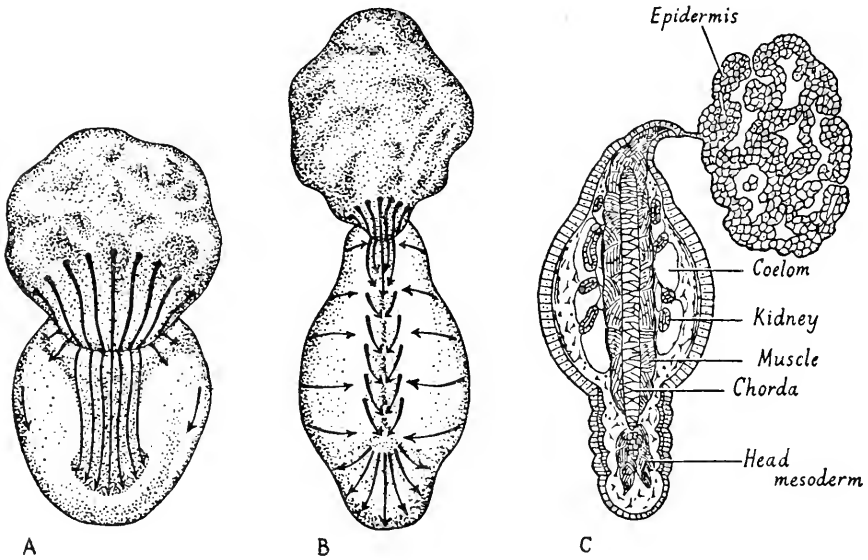


FIGURE 20.12

A, *B* are drawings of two stages of exo-gastrulation in the axolotl; the ectoderm lies above, and the endo-mesoderm, instead of moving inside it, is elongating towards the bottom of the picture. *C* is a diagrammatic section through a later stage. (After Holtfreter 1933.)

The second factor has attracted considerably more study than the first. It can fairly easily go wrong if embryos, particularly of the axolotl, are allowed to gastrulate in solutions of abnormal osmotic pressure. There is then often a failure of the mechanism by which the elongating dorsal material should be turned inwards, and part of the mesoderm and endoderm (or even the whole of it) may move outwards from the blastopore, forming a so-called exogastrula. In less strongly affected cases it is the anterior mesoderm which fails to get inside, while the more posterior

material does so. Holtfreter (1933) has described the process in detail. He showed that the regions of the neural system developed in such partial exogastrulae correspond very closely with the extent of the mesoderm that is formed inside as opposed to outside, a fact which provides a neat illustration of the developing pattern of anterior and posterior organisers, which we shall shortly discuss (p. 455) (Fig. 20.12).

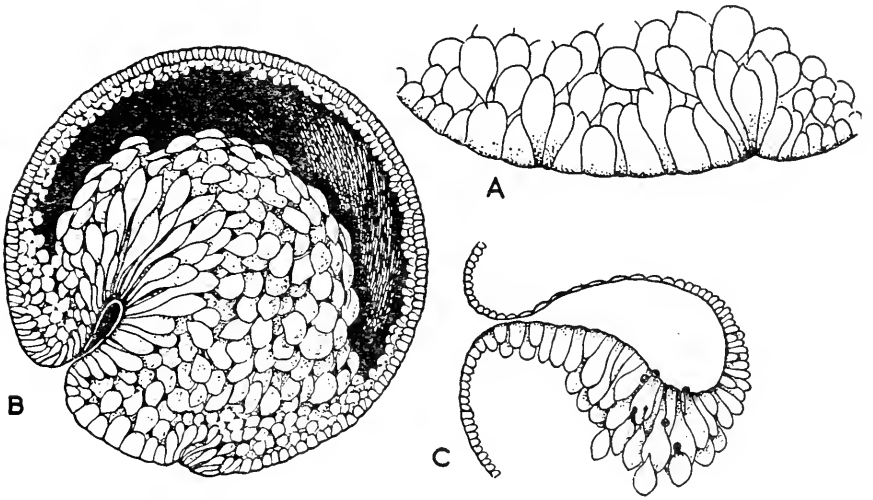


FIGURE 20.13

A, section through the future blastopore region of the axolotl, at a time when the invagination is indicated only by condensations of pigmented coat to which early flask cells are attached. *B*, semi-diagrammatic section through a gastrula, to show the flask cells lining the blastopore and archenteron. *C*, the endoderm cells forming the floor of the archenteron at a later stage; cells which have broken contact with the surface have contracted their 'necks' into small pigmented lumps. (From Holtfreter 1943.)

There are at least two factors involved in the in-turning mechanism. The most obvious is the formation of peculiarly shaped cells at the position at which the blastopore first appears. These are usually described as flask or bottle cells. The main cell body, in which the nucleus is situated, is roughly ovoid and is drawn out into a long, thin neck by which it is connected to the external surface in the blastopore region. The narrowness of the neck may be partly the result of the contraction of the coat over the site of the blastopore furrow, but it is probably also influenced by processes going on within the cell cytoplasm, since the neck region, from which yolk granules are absent, has been found to show double

refraction which would indicate the appearance of a fibrous structure of the cytoplasm (Waddington 1940). The neck is certainly very contractile, and its tendency to contract almost certainly plays a part in pulling the elongating dorsal material inwards and thus beginning the gastrulation process (Fig. 20.13).

A movement of one piece of tissue towards the interior of another tissue mass can, however, occur with little sign of the participation of flask cells

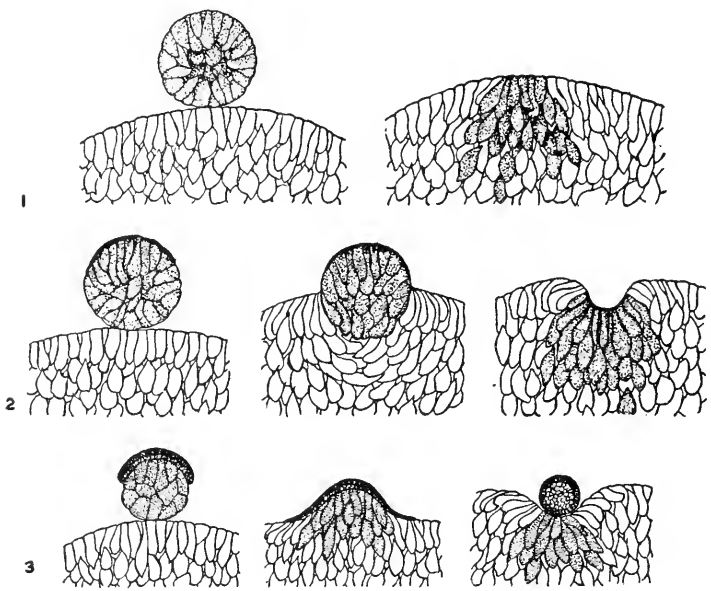


FIGURE 20.14

Engulfment by the endoderm. Row 1, a small fragment of uncoated endoderm becomes incorporated into an endodermal substratum. Row 2, a fragment of blastoporal cells, partly covered by coat, behaves similarly but also forms a groove. Row 3, if the endodermal fragment is covered by ectoderm, the latter at first spreads but then rounds up and becomes isolated. (From Holtfreter 1943–44.)

in the procedure. Holtfreter (1943–1944) has described how groups of blastoporal or endodermal cells, placed against a larger mass of endoderm, become as it were engulfed into it (Fig. 20.14). He has shown that important factors in this situation are the adhesiveness between the membranes of different types of cells, and what he speaks of as the 'surface tensions' developed along such cell-to-cell or cell-to-medium interfaces. By placing in contact small groups of cells from different tissues, he was

able to show very clearly that the adhesiveness of a cell for other cells of the same or different kinds may change considerably during the course of differentiation. Thus in some tissues the cells are very closely packed at certain stages, when their adhesiveness is high, but tend to become dissociated from each other at other times, as for instance when the neural crest cells break away and start to migrate separately between the ectoderm and mesoderm. Again, combinations of different tissues sometimes show considerable mutual affinity, when they round up into a single mass, while at other stages the two tissues may tend to separate from one another (Holtfreter 1939). Such processes are undoubtedly very important in influencing the shapes of neighbouring masses of tissues in a developing embryo. Holtfreter has given a number of diagrams illustrating the effects of these mutual interactions on the forms assumed by tissues some way along in their differentiation (Fig. 20.15).

At the stage of gastrulation, differentiation has not proceeded very far and one would not expect to find clear-cut differences in affinity, but

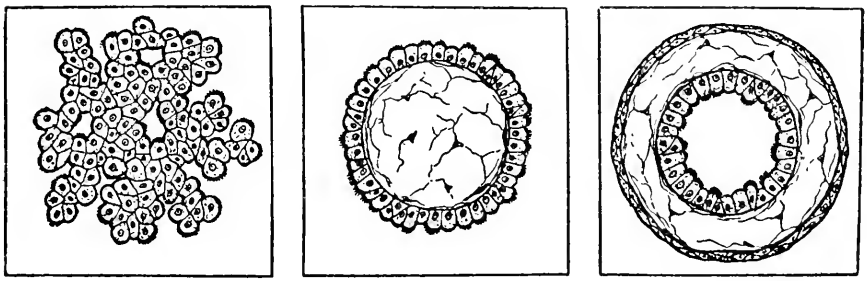


FIGURE 20.15

Figure *a*, isolated ectoderm develops into an irregular epidermal tissue; *b*, ectoderm combined with some mesenchyme forms an epithelium ciliated on the outer surface; *c*, ectoderm embedded in mesenchyme, inside another epidermal skin, forms a vesicle or tubule ciliated on the inner surface. (From Holtfreter 1939.)

instead more gradual transitions between one region and another. Holtfreter gives some evidence that such graded differences exist. He is inclined to interpret them as differences in surface tension. He points out that if one has two drops of different materials, *A* and *B*, in contact and both immersed in a medium *C*, and if the tension in the *AC* surface is greater than the sum of the tensions in the *BC* and *BA* surfaces, then the *AC* surface will contract, and the drop of *A* will in effect be engulfed by

the drop of *B*. This, Holtfreter suggests, may provide a model of the incorporation of groups of cells placed in contact with endoderm, and indeed of one of the factors involved in the normal invagination (Fig. 20.16).

The general principle of the suggestion seems rather plausible, but some caution is advisable in using the term 'surface tension' in connection with amphibian cells. Strictly speaking, a surface tension develops only in a liquid interface, and there seems little doubt that the external membranes

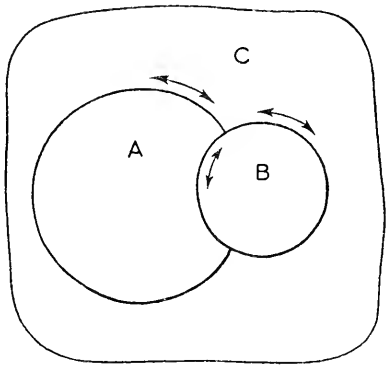


FIGURE 20.16
Surface tensions in two drops of different fluids *A* and *B* immersed in a third *C*.

of cells cannot be regarded as truly liquid, but must be supposed to possess a certain degree of rigidity or solidity. This does not make it impossible, however, for such surfaces to exhibit properties analogous to those of the tensions which would develop in truly liquid interfaces. Returning to the two drops *A* and *B* in contact with one another, the engulfment of *A* would also occur if there was a strong tendency for the area of contact between *A* and *B* to increase, and such a tendency would arise if the *A* and *B* membranes in some way attracted one another. Since the membranes are largely protein, they may be expected to exhibit an orderly disposition of chemically reactive groups, which will tend to become attached to the appropriate groups on some neighbouring surface if the two patterns fit, but not otherwise.

Weiss (1947, 1949, 1950*b*) has emphasised the important part that may be played in development by the mobilisation at the surface of the cell of compounds that have a specific chemical reactivity which causes

them to enter into combination with substances at the surface of other neighbouring cells. He has laid particular stress on the influence of such reactions in changing the constitution of the internal cytoplasm by anchoring certain constituents on the surface, and he suggests that this may provide a general mechanism of differentiation (Fig.19.3). It is not easy to admit the general importance of the hypothesis in connection with the differentiation of substance, if only because differentiation can proceed quite well in unicellular Protozoa, or in completely isolated amphibian notochord cells (Mookerjee 1953), or pigment-forming cells (Twitty and Niu 1954). But processes of this kind may play a major part in morphogenesis. Schmitt (1940) has also discussed, in what one might call generalised chemical terms, how cell surfaces might be bound together by a an intermediate layer which could react with both of them (Fig. 20.7).

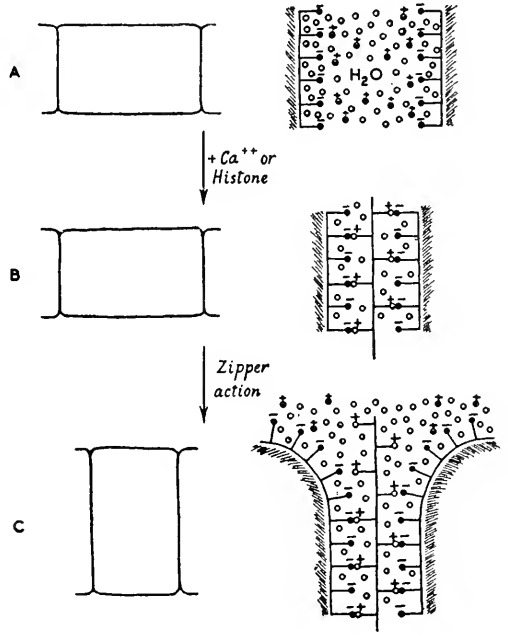


FIGURE 20.17

A represents a rather flat cuboidal cell; its surface where it makes contact with the next cell is highly solvated, and adhesion between the cells is slight. If a desolvating agent (calcium ions, or histone) is introduced, the cells will be drawn together as though by a zipp fastener, eventually (*C*) forming tall cells with considerably greater surface of mutual contact. (From Schmitt 1941.)

Whatever the detailed chemical mechanism, it seems legitimate to think of the membranes of cells which are in contact with one another as behaving as though they were being forced together, or forced apart, by a number of zipp-fasteners, each with a characteristic tension tending to extend the zipped region. Such a system would behave very like a group of cells with truly fluid membranes possessing true surface tensions.

It is very probable that forces of a similar kind, arising from the adhesiveness of the cell membranes, play a part not only in the in-turning of the invagination stream but also in producing it. It is certain that during the elongation and narrowing of the dorsal material, and the compensating expansion of the more ventral parts of the gastrula, there are considerable changes in the mutual contacts between the cells. One evidence of this is the fact that the layer of tissue forming the blastula roof, which is several cells thick at the beginning of gastrulation, is reduced to a thickness of little more than two cells by the time gastrulation

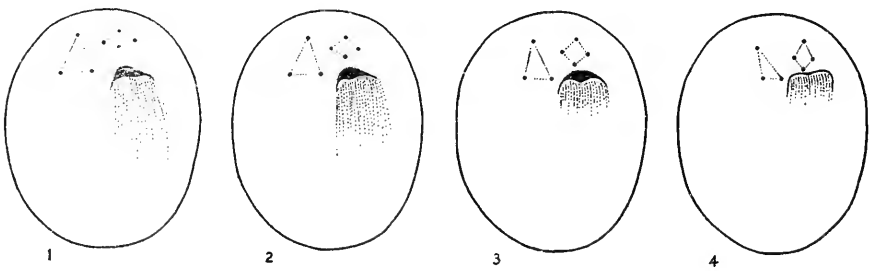


FIGURE 20.18

Four successive stages in gastrulation in *Triturus alpestris*. A group of four, and a group of three, cells have been followed, to show the changes in arrangement of the cells accompanying the streaming towards the blastopore tip. (Original, from a time lapse cine film.)

is completed. Again, if cells are watched approaching the blastopore lip it will be found that they have slid in between one another in such a fashion that a group originally arranged in lines lying parallel to the blastopore lip has become rearranged into a longer and narrower shape (Fig. 20.18). A similar interdigitating movement of cells continues on the floor of the neural groove. The elevation and folding together of the sides of the groove may, in fact, be partly caused by the continuous anterior-posterior stretching of its midline, just as the edges of a piece of rubber will roll up on each side of a line of stretch.

Anterior-posterior stretching continues also in the internal dorsal material, namely the presumptive notochord. One could explain the whole morphogenesis of the presumptive mesoderm, into a central notochord flanked by rows of somites, in terms of tendencies for cells to move together in particular regions (Fig. 20.19). We might suppose that, as the mesoderm develops, there is at first a very strong tendency for cells near the midline to increase their surfaces of mutual contact and thus to interdigitate and form a narrow coherent rod. If this adhesiveness fell off rapidly from the midline towards the sides, cells slightly lateral to

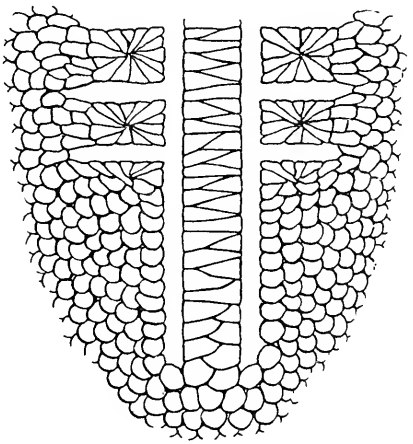


FIGURE 20.19
Diagram of cell arrangement in the mesoderm of the newt neurula, showing the developing notochord and somites.

the midline might have such a strong tendency to move towards it that they were pulled out of contact with cells lying further away; and thus the notochord would be isolated as a long rod of tissue separated from the more lateral mesoderm by a gap. To explain the next stage, the formation of the somites, one would have to suppose that a tendency for cells to move closer together begins to appear in more lateral regions, being at first stronger in the anterior and falling off rapidly towards the posterior. Then a transverse split would appear behind the first group of aggregated cells, isolating the first pair of somites; and if the process gradually spread backwards, the whole series of somites would be successively formed.

The tendencies of cells to move together, which have been postulated in the above scheme, are in most cases real enough, as can be seen by examining the embryos at various stages. The hypothetical element is the suggestion that these tendencies for particular types of aggregation are produced by forces arising from the adhesiveness of the cell membranes. Again, we know that such forces exist in isolated cells (cf. Holtfreter 1947,

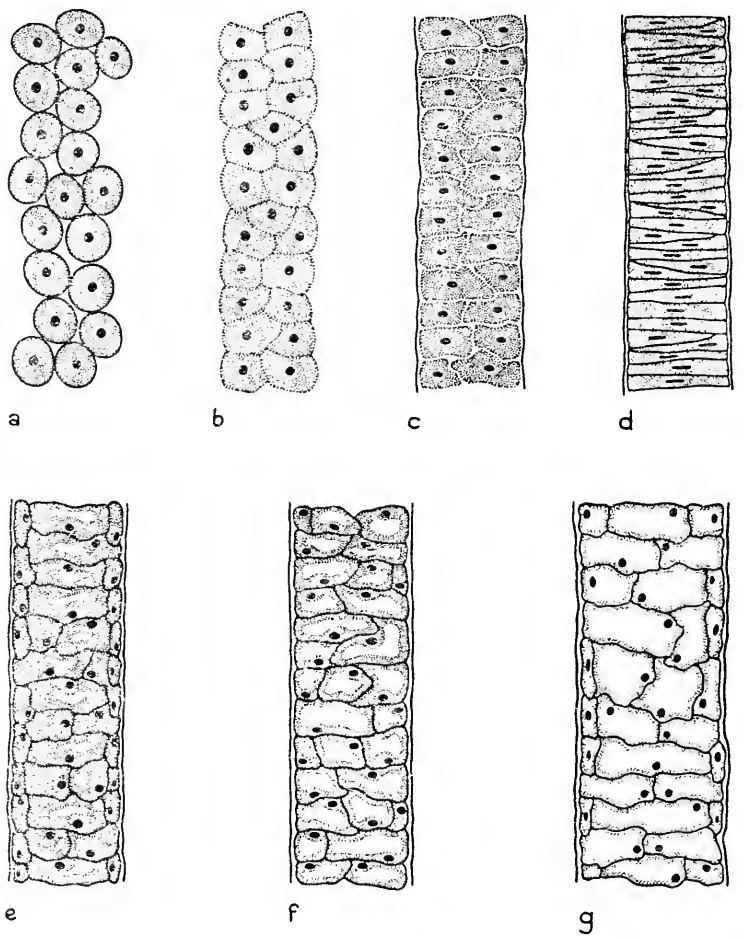


FIGURE 20.20

Stages in the development of the rounded cells of the archenteron roof into the notochord in the newt. At stage *b* the cell boundaries are difficult to see; at *c* the chordal sheath begins to be formed. The whole process of development consists, in the main, in an increase in the relative extent of the cell surface. (From Mookerjee, Deuchar and Waddington 1953.)

1948*a*). But are they operative among the cells of the gastrula? In most phases of gastrulation and neurulation we still have not enough detailed knowledge of the shapes of the cells adequately to judge the plausibility of this. One process has, however, been examined in some detail from this point of view, namely the formation of the notochord in the midline of the invaginated mesoderm (Mookerjee, Deuchar and Waddington 1953). It is quite clear here that the area of contact between cells does increase considerably as the notochord forms. In fact the cells of the notochord soon take up an arrangement which has been compared to a pile of coins, each cell being a more or less flat disc with the maximum possible contact with other similar cells. The later stages of differentiation of the notochord, in which the cells become vacuolated until they are mere distended bags full of cell sap, might be regarded as a consequence of the transformation of more of the original cytoplasm into membrane, so as to increase still more the area of intercellular adhesion (Fig. 20.20). In this example the increase in cell contact is clearly one of the basic phenomena of morphogenesis, and it seems not unreasonable to accept it as a causal explanation of the events. Forces arising from the cell membranes may well be the prime cause of the changes in tissue configuration during the whole process of gastrulation and neurulation (Fig. 20.21).

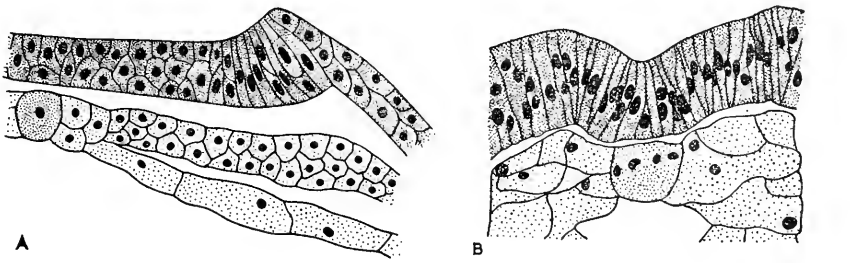


FIGURE 20.21

Semi-diagrammatic transverse sections of the neural plate of the newt. In *A* the edges of the plate are only just beginning to fold upwards; the central part of the plate is occupied by nearly equidimensional cells, while at the edge the cells have become columnar and greatly increased the area of cell-to-cell contact. *B* shows the centre of the plate at a rather later stage, when cells have all become elongated. (*B* from Lehmann 1945.)

It must not be supposed, however, that cell-membrane forces are always the most important factors in causing morphogenetic change. Elaborate and definite shapes may indeed be assumed by single cells, as for instance in nematodes where the greater part of the excretory canal forms within the body of one cell.

There is still one aspect of gastrulation movements which remains to

be discussed; that is the fact that they are strongly polarised. It was recognised by Spemann in the early experiments with organisers that if tissue from the blastopore region is grafted into another site in an embryo it tends to go on invaginating in its own original direction, although it very often becomes swung round into conformity with the gastrulation movements of the host. Particularly striking examples of the tendency of a graft to retain its own polarity and direction of invagination can be seen in anurans, such as *Discoglossus*, in which development is very rapid and the anterior-posterior elongation and lateral narrowing of the blastopore material particularly well marked (Waddington, 1941). It is still rather unclear whether this polarity is a property of pieces of tissue, i.e. of groups of cells in which, perhaps there is a gradient in the intensity of the cell-membrane forces, or whether it is inherent in the individual cells of which the tissue is composed. Holtfreter (1947) has given a somewhat diagrammatic drawing of the recently invaginated mesoderm, in which a polar structure of the individual cells is indicated, and he has also shown experimentally that isolated cells do develop a polarity of their own. However, if this cell polarity exists before gastrulation it is certainly by no means irrevocably fixed, since small parts of the presumptive mesoderm of the newt, cut out and replaced with the anterior-posterior axis reversed, may in some cases invaginate in perfect conformity with their surroundings and show no signs of reversed cellular polarity (cf. p. 458).

5. *Measurement of the forces and energy involved in morphogenesis*

During morphogenetic changes regions of tissue are shifted about bodily in space; that is to say, work is done, and energy must be expended. Many attempts have been made to divide the energy used by an embryo into a fraction required for simple maintenance of the living system, and another fraction devoted to the performance of this morphogenetic work. In practice it has been found extremely difficult to do this; the earlier work on the subject has been fully discussed by Needham (1931).

It has indeed been difficult even to demonstrate the existence of a morphogenetic energy fraction, let alone to measure it. One of the most successful attempts to do so has been that of Tyler, summarised in his review of 1942. He compared the rates of development and the oxygen consumption of embryos derived from whole echinoderm eggs or from separated first blastomeres (half embryos) or from fused eggs (giant embryos). He found that the rate of respiration was nearly the same in all, but that half embryos took a longer, and giant ones a shorter, time to reach a given stage than did normals. Thus by the time they reach a given stage, the half embryos have consumed more and the giants less energy

per unit mass than the normals. Tyler argues that this is a reflection of the varying amounts of morphogenetic work per unit mass which the different types have to perform. This may indeed well be so; but, as Needham (1942) points out, the slow-developing half embryos have not only got to do twice as much morphogenetic work per unit mass as the normals, but also have to maintain themselves for longer before reaching any particular stage; and it remains obscure how much of the extra energy goes to one purpose or to the other.

If considerable quantities of energy were utilised for the performance of physical work, then there should be a measurable discrepancy between the decrease in the calorific value of an embryo as its yolk is consumed and the amount of heat which it gives out. The most thorough study of this question is that of Smith (1946), and no such difference was found. Various other authors have attempted to estimate the maximum fraction of the utilised energy which can possibly be supposed to be devoted to such work. Tyler gives a figure of 30 per cent for echinoderm eggs. Tuft (1953) has reviewed the measurements on the rate of oxygen consumption which have been made on the developing eggs of a number of different species (insects, fish, Amphibia). He shows that the curves are often by no means simple, but may have a succession of phases of increasing, stationary or even decreasing rates. In the bug *Rhodnius*, the rate of oxygen consumption falls during a certain period. Tuft makes a calculation, based on the supposition that the minimum of the rate indicates the maximum consumption which can be considered necessary for maintenance, and concludes that it is conceivable that as much as 15 per cent could be devoted to something else, such as morphogenetic work. The weak point in such an argument is of course the assumption that the maintenance requirements remain constant (Fig. 20.22).

Little is as yet known about the biochemical systems by which energy is delivered to the morphogenetic mechanism; probably they involve high-energy phosphate compounds (Barth and Barth 1951). The ease with which a process such as gastrulation is brought to a standstill (e.g. by thermal shocks, a wide range of chemical inhibitors, etc.) suggests that the process is a very sensitive one. The ultimate source of the energy for amphibian gastrulation is presumably carbohydrate, since, as we have seen (p. 203), the consumption of glycogen increases greatly in the blastopore region just when movement begins. It may well be, however, that other morphogenetic processes obtain their energy from other sources. Needham (1931) claimed that it is a general rule that during embryonic life, the predominant source of energy is, in the earliest stages, carbohydrate, then protein and finally fat. More recent investigations (e.g.

Løvtrup 1953) show that, in Amphibia at least, the succession is really carbohydrate-fat-protein. Gregg and Ornstein (1953) found that certain of the morphogenetic processes studied by Holtfreter in explants (cf. p. 444) were more sensitive to one group of enzyme inhibitors, others to different ones, which suggests that they do not all derive their energy in the same way.

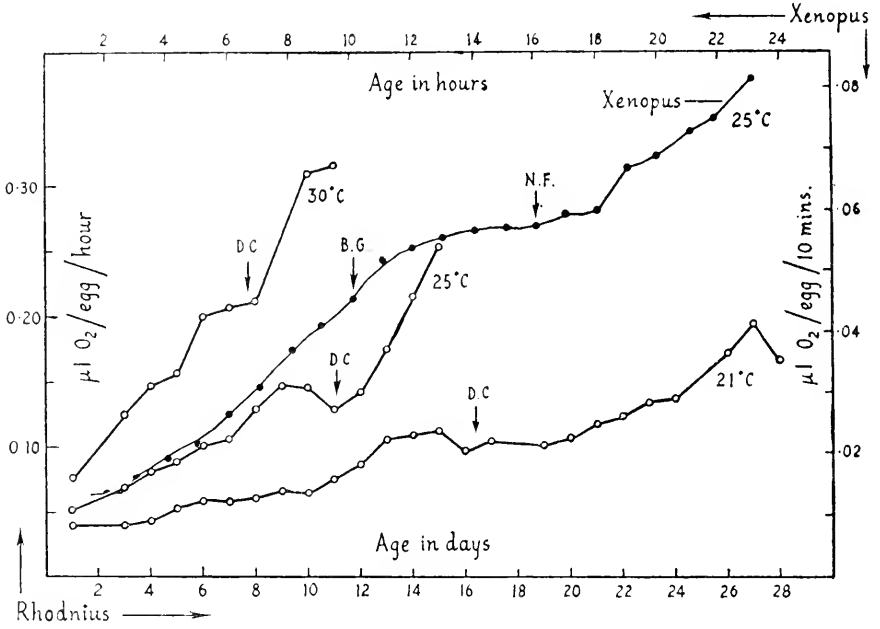


FIGURE 20.22

Changes in the rate of oxygen consumption during the development of single eggs of the bug *Rhodnius*, at three different temperatures (*D.C.*, time of dorsal closure; scale at left and below). And the same for the anuran *Xenopus* (*B.G.*, beginning of gastrulation, *N.F.*, neural fold stage; scale above and to right). (After Tuft 1953.)

A more direct approach to the problem of the energetics of morphogenesis would be to measure the actual forces which are exerted. In one attempt to do this for amphibian gastrulation, a small steel ball was inserted among the cells of the invaginating mesoderm, and a bar magnet placed nearby in such a position that the magnetic force on the ball tended to prevent its movement. From a series of such measurements, one can arrive at a rough figure for the maximum force which the gastrulation movements can exert. This was found to be about 0·34 dynes per mm.^{2} of the hemispherical surface of the ball (Waddington 1939*b*).

Selman (1955) has made similar measurements of the force of neuralisation; his method consisted in placing two minute steel dumbbells against the two neural ridges, and holding them apart by placing the whole preparation within a coil carrying an alternating current; he found a value of 40×10^{-3} dynes. Using an estimate of the distance over which the tissues move, one can roughly estimate the total amount of work accomplished: during gastrulation it is about 3×10^{-9} cal. per hour. Even if one supposed that the energy-producing mechanism was working at an efficiency of only 10 per cent, the amount of oxygen consumed during the period could produce about a million times as much energy as this, so that these figures suggest that morphogenetic work demands only a very minute fraction of the energy available. It should be emphasised, however, that the measurements of the forces are of a very preliminary character; they may be too small, because the cells have been damaged during the experiment, or alternatively they may be larger than the forces actually exerted, since the measurement is of the maximum the tissues can put out rather than what they normally do.

A measurement of the force of gastrulation has been made by another method in echinoderms by Moore (1941, 1945). If an echinoderm egg is cultured from fertilisation onwards in sucrose solution, some of this becomes enclosed in the blastocoel, but when this cavity is fully formed the solution cannot escape since the walls of the blastula are impermeable to the sugar, which therefore exerts an osmotic pressure, directed outwards, if the blastula is transferred to sea water. By finding what concentration of sucrose just prevents the in-pushing of the endoderm, Moore decided that the force of gastrulation in this form is about 5 gm. per mm.^{2}. This is about 10^4 as great as that found for the very different amphibian gastrulation process. Even so, as Moore points out, the work done in echinoderm gastrulation would only demand about one thousandth of the oxygen which is actually consumed.

6. *Individuation of the central nervous system in Amphibia*

The development of the nervous system from the gastrula to the neurula stage in Amphibia provides probably the best example from which one can get an idea of what is actually involved in the individuation of most embryonic organs. Not only has the process been very thoroughly studied in a long series of experiments by many authors, but it involves simultaneously both the aspects of individuation—morphogenesis and pattern formation—which we have just considered separately. In reality they must usually occur in combination with one another, and the development of the nervous system therefore gives a more generally valid picture

than the rather special cases we have chosen to exhibit the details of each process separately. Moreover, in the individuation of the nervous system a process of induction is very clearly involved and the pattern of the nervous system itself is partly derived from that of the underlying mesoderm. This is perhaps rather a special feature which is not found, at any rate with such clarity, in the development of many other organs, but it has the advantage that it offers opportunities for experimental analysis which would not otherwise be possible.

By the time the blastopore has become reduced to a narrow slit, gastrulation may be said to be complete (although in the immediate neighbourhood of the blastopore invagination of mesoderm will continue for some time longer). The structure of the sheet of mesoderm which acts as the inducer of the neural plate is at this time as follows. At its most anterior end it is thin and stretches widely from side to side; this part lies in front of the future notochord and is known as the prechordal plate. Posterior to it is the main mass of the chorda-mesoderm, the dorsal part of which will become notochord, the lateral part somites, with the intermediate mesoderm and the side-plate mesoderm still more laterally. Overlying the whole mesoderm is the ectoderm on which a neural plate is, or shortly will be, delimited by the appearance of the neural ridges. From anterior to posterior the neural plate can be divided into four main regions. The most anterior of these, which overlies the prechordal plate, will become the forebrain (which is also commonly known as prosencephalon, but by some authors (e.g., Lehmann) as the archencephalon, and by others (e.g., Dalcq) as the acrencephalon). This develops into the two vesicles of the forebrain, the second of which bears the eyes, and it becomes associated with the nasal placodes. Posterior to it is the region which will become the midbrain and hindbrain which are collectively known as the deuterencephalon and are associated with the ears. The anterior tip of the chorda lies somewhere within this region. Further posteriorly is the spinal or trunk region; and finally the most posterior end of the neural plate consists of material which is not truly neural at all but will form part of the mesoderm of the tail. The neural plate also has a certain structure in the transverse plane. In the dorsal midline the tissue is rather thin, forming a shallow groove. On each side of this lies the main bulk of the neural plate, which will form the walls of the neural tube. At the two edges are the neural ridges which will develop into the neural crest and eventually form pigment cells, parts of the spinal nerves and certain ectomesodermal derivatives, such as some of the cartilages of the head.

The question immediately arises how the pattern of regions arising within the neural plate is related to patterns which may be present in the

inducing mesoderm. Spemann (1931) pointed out that the inductive capacities of the different regions of the mesoderm were not all the same even at the beginning of gastrulation. The presumptive anterior regions tend, other things being equal, to induce more anterior parts of the neural plate than do presumptive posterior regions. Shortly after this, Mangold (1933) showed that if different regions from anterior to posterior are cut out from the archenteron roof and implanted into young gastrulae they show characteristic differences in the region of the nervous system which they induce. It seemed then that one could consider the mesoderm from the early gastrula stage onwards as having a fairly high degree of regional specificity, so that the presumptive anterior part could be considered as a 'head organiser' and the presumptive posterior part as a 'trunk or tail organiser'.

In agreement with this Hall (1937) showed that if the blastopore lip is removed from a young gastrula and replaced by the lip from a considerably older gastrula with a yolk plug, this presumptively posterior graft failed to induce a head, the neural system of the resulting embryo having a spinal character right to its anterior tip. Holtfreter (1936) has given a map of these head organisers and trunk organisers (Fig. 10.3, p. 178). Later authors have claimed that there are more than two regions—the head and trunk—which behave independently of one another. Lehmann (1945) and Dalcq (1947) have both argued that one must consider at least the three main regions mentioned above, namely the forebrain, the mid-brain, and hindbrain and the trunk regions. Nieuwkoop (1947) presents evidence that in secondary induced embryonic axes the brain is always fully formed up to a certain level, anterior to which it is altogether absent, and on this basis he distinguished at least seven successive independent zones.

The experimental results always made it clear that the regional specificity of an organiser was not something absolutely fixed in the sense that the organiser could induce only one specific region of the neural plate and nothing else. The results indicated at most a tendency for the induction to have a certain regional character, but trunk organisers, for instance, could sometimes induce more anterior parts and vice versa. These disturbances in the simple picture can be to some extent accounted for, at least in experiments in which the organisers are grafted into whole embryos, by the supposition that the region of the host embryo in which the graft lies exerts an influence on the regional character of the material induced. However, a certain degree of latitude in the specificity of a particular region of the mesoderm is found even when it induces in isolated pieces of ectoderm removed from the influence of any host

embryo. (Extensive studies on regional specificity during gastrula stages have been made by Okada and a group of Japanese workers (see Hama 1950), but seem to suffer from lack of adequate statistical evaluation.) One is forced to admit that the regional character of the various parts of the mesoderm is only imprecisely determined during the process of gastrulation and is still to some extent labile.

This is indeed what one might expect from other types of experimental evidence. Thus, as pointed out earlier (p. 190) Yamada (1940) has shown that even in the neurula the 'level' of the mesoderm on the dorso-ventral axis is not yet finally fixed; for instance, presumptive lateral plate can be forced to develop into somitic muscle if notochord is brought into its neighbourhood. A similar flexibility occurs along the anterior-posterior axis. Waddington and Yao (1950) showed that if presumptive anterior or posterior portions of the organiser are exchanged in young newt gastrulae, completely normal individuals may be produced, which must involve an alteration in the anterior-posterior specificity of the exchanged region. (In similar experiments with the rapidly developing gastrula of the anuran *Discoglossus* [Waddington 1941] the morphogenetic tendencies of a graft were so strong that they prevented its incorporation by the host and no redetermination of the regional character could be proved in that case.) Even at the end of gastrulation considerable flexibility still persists along the anterior-posterior axis, since a fairly normal embryo (with over-thick mesoderm) may develop if an extra archenteron roof is added with reversed orientation between the normal archenteron roof and the presumptive neural plate (Fig. 20.23).

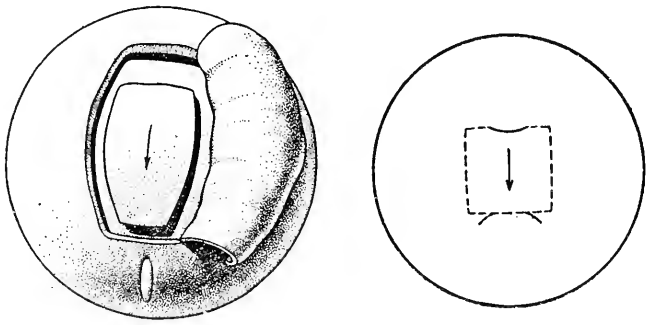


FIGURE 20.23

Some experiments on the regional properties of the organiser. On the left, the presumptive neural plate of a late gastrula folded back, a second archenteron roof laid with reversed orientation over the original roof, and the ectoderm returned to place. On the right, reversal of the dorsal lip region. (From Waddington and Yao 1950.)

Even at the neural plate stage, the regional specificity is not completely fixed, either in the neural tissue or in the mesoderm underlying it. This was clearly shown in extensive experiments by ter Horst (1948). She separated the neural plate from the archenteron roof, cut each into five transverse strips, and cultured these after wrapping them in pieces of young gastrula ectoderm, and observed both the differentiation of the isolate and the character of the induction it produced. The neural plate fifths on the whole developed into their presumptive fate, but showed a tendency to produce the next most anterior and most posterior regions as well—thus they still have a capacity to regulate towards the formation of a more complete neural system. The differentiation of the mesoderm showed evidence of another kind for a lack of full determination—the anterior parts of it sometimes developed some neural cells. In their induction effects, the two tissues gave even more evidence of flexibility, each fifth inducing a certain region in greatest frequency, but also calling forth neighbouring regions in considerable numbers (Fig. 20.24). A remarkable fact is that a given fifth of the archenteron roof tends to induce

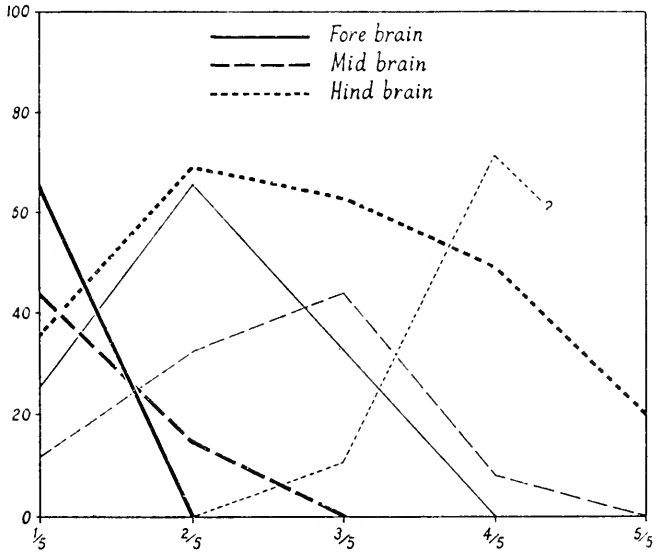


FIGURE 20.24

The open neural plate of the newt is divided into five equal zones, from anterior (1/5) to posterior (5/5); the neural plate is dissected free from the archenteron roof; and neural plate and mesoderm are then separately combined with flaps of young gastrula ectoderm. The thick lines show the frequency of inductions of fore-, mid-, and hind-brains by the neural plate, and the thin lines that by the mesoderm. Note that the neural plate has a more 'anterior' performance than the corresponding mesoderm. (After ter Horst 1948.)

more caudal regions than does the overlying part of the neural plate. This may perhaps find its explanation in Nieuwkoop's hypothesis of a second phase of the induction process, in which the induced material is gradually transformed into more posterior regions of the axis (p. 462).

We find, therefore, that during the process of gastrulation, while the mesoderm is exerting its inducing influence, it is itself only just in the process of acquiring a pattern of regional specificities. It is these half-formed specificities which must be responsible for the regionally different types of induction which the various areas of the mesoderm exert. The main question which has been debated recently is whether the specificities within the mesoderm, and the various types of induction for which they are responsible, are to be explained in terms of quantitative variations in the concentration of some one substance, or whether the facts require us to consider that different substances are being produced, characteristic of the different regions. One may probably assume that by the time histological differences can be recognised within the mesoderm sheet the various regions have come to be characterised by particular substances. We have seen (p. 216) that investigations on the inductive capacities of different adult tissues have led to the conclusion that there are at least two different inducing substances, one for forebrain and another for trunk-tail (or perhaps solely for mesoderm). The question is, have such chemical differences arisen already at the time of gastrulation when the induction of the neural plate first occurs, or is the individuation of that original induction dependent only on a pattern of quantitative differences in the mesoderm?

It does not seem possible at present to give a perfectly firm answer to this question. Most authors, however, seem to be agreed that the mesoderm behaves as though composed of at least two different systems, one corresponding to the prechordal plate and the other to the main mass of chorda-mesoderm. It is to be presumed that these are chemically distinct, but there is some divergence of opinion as to exactly what effects the two regions produce, and as to the importance of quantitative variations within them. Dalcq (1947) has recorded a series of investigations in which the young gastrulae of *Discoglossus* were cut into two portions along some parallel of latitude which separated the organisation centre into a more animal (presumptively posterior) portion and a more vegetative presumptively anterior portion. The animal part is then rotated through 180 degrees and replaced on the lower in such a way that the posterior organiser it contained lay on the ventral side (Fig. 20.25). Both parts of the organiser invaginate and induce partial embryos. From the study of such incomplete embryos, Dalcq came to the conclusion that the prechordal plate always induces the forebrain or associated structures, and

that its effects can be arranged in a series of grades corresponding to the amount of inducing material present. The lowest grade of induction, he claims, is an isolated pineal body. When larger quantities of prechordal plate are present a small forebrain vesicle is induced. The next stage induces the appearance of a finger-like anterior expansion which is capable of inducing an olfactory placode. With still more intense inductive action the eyes appear.

Dalcq suggests that similar variations in effectiveness, dependent on quantitative differences in the inducer, occur also in more posterior parts

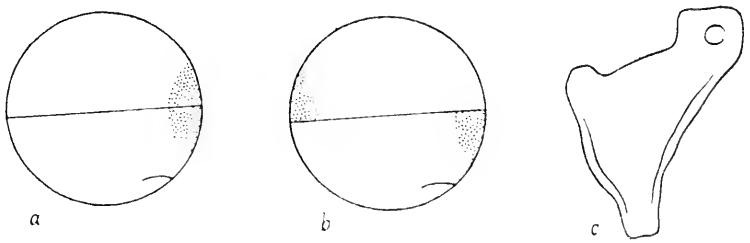


FIGURE 20.25

Figure *a*, a young gastrula of the anuran *Discoglossus* is marked with a vital stain above the blastopore, and then cut in two along a parallel of latitude; *b*, the upper half is rotated through 180 degrees and replaced; *c*, a normal embryo is usually formed on the original dorsal side (unless the cut was too low), and a partial embryo, lacking some part of the anterior region, appears on the ventral side. (After Dalcq 1947.)

of the nervous system overlying the chordamesoderm. The most clear-cut evidence in regard to these regions concerns the medio-lateral extent of the plate rather than its anterior-posterior structure. There is considerable evidence that the inductive capacity of the chorda-mesoderm is most powerful in the midline and decreases on either side. Thus it can be shown that at the late gastrula stage the lateral plate mesoderm has a weak capacity of induction which can be effective on the highly reactive ectoderm of the young gastrula stage, but not on the less-reactive ectoderm of older gastrulae (Waddington 1936*a*). Raven and Kloos (1945) have made grafts from medial or lateral parts of the archenteron roof underlying the neural plate and showed that the lateral ones were weaker inducers. Again, two of Dalcq's students, Damas (1947) and Gallera (1947), have studied the effects of cutting short the period in which induction can proceed. At

various times after the presumptive neural plate had been underlain by mesoderm, they removed small fragments of the plate, cleared them of adhering mesoderm cells and transplanted them to the ventral side of another embryo. They found that ectoderm which had been acted on for only a short time tended to differentiate into neural crest rather than into neural material proper. There seems little doubt then that neural crest presents a weaker grade of induction than neural tissue. These experiments do not, however, provide direct evidence that differences along the anterior-posterior axis (e.g. differences between midbrain and hindbrain, trunk, tail, etc.) also represent a series of grades of the strength of the inducing action.

A somewhat different account of the action of the prechordal plate and the chorda-mesoderm has been given by Nieuwkoop (1952). He had the ingenious idea of joining elongated flaps of ectoderm to the gastrula in such a way that the base was near the archenteron roof, but there was quite a long extent of ectoderm into which the inducing material could diffuse. He found that in general the regional character of the structures induced at the base of the ectoderm flap was the same as that of the region to which it was joined, and that the induced structures extended from there towards the forebrain, which might be located at the free end of the process of ectoderm, although sometimes the inductions were incomplete and lacked the most anterior regions. Nieuwkoop interpreted his results by the hypothesis that all parts of the archenteron roof (chorda-mesoderm as well as prechordal plate) exert a first inducing action which stimulates the ectoderm to develop into forebrain-like organs; after this has occurred the chorda-mesoderm, if it reaches the ectoderm in question, exerts a second 'transforming' activity which changes the development of the induced material towards the production of some more posterior level of the axis (Fig. 20.26).

Such a hypothesis fits in well with the ideas developed by Toivonen from his studies on the inducing powers of adult organs (p. 216). However, one cannot help feeling that the results described by Nieuwkoop scarcely prove his hypothesis. The phenomena in the ectoderm flaps, which take on the regional character of the point at which they are attached and exhibit the structures which would normally lie anterior to this, remind one very much of the appearances in a limb regenerate, the base of which develops the regionality of the cut face on which it forms and which contains all the structures lying distal to this. In the case of the limb regenerate, it scarcely seems plausible to suggest that the first phase is the determination of the distal tip of the limb, and the second phase a transformation into more posterior regions; and such a hypothesis, though

perhaps more plausible in Nieuwkoop's case, does not seem by any means necessitated by the data.

His hypothesis is, however, supported by the recent work of Eyal-Giladi (1954), who claims to have shown that when any part of the mesoderm acts on ectoderm the first result is to confer on the latter the capacity to differentiate into neural crest, but that this stage is fairly rapidly passed through, and in the next period the ectoderm is always induced to form

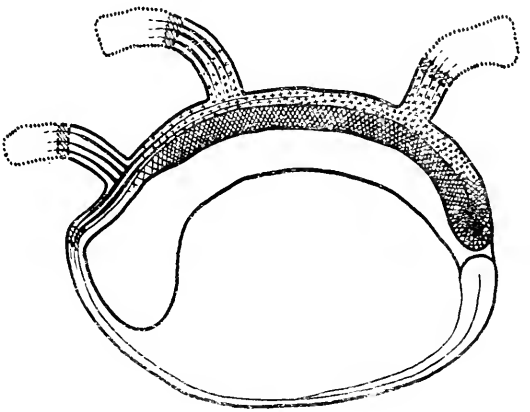


FIGURE 20.26

Flaps of competent gastrula ectoderm are grafted on to a host embryo in such a way as to become attached to the forebrain, hindbrain or spinal cord. The differentiation of the flaps is indicated by: dotted outline, epidermal; cross-hatched, mesectodermal; longitudinal lines, forebrain; crosses, more posterior neural regions. The diagram illustrates Nieuwkoop's interpretation, which supposes that at first the whole of the induced region acquires a tendency to form forebrain, and that in the more posterior flaps this is later transformed towards more spinal development. (From Nieuwkoop 1952.)

forebrain, while if the inductive action lasts even longer the transforming action will cause the production of more posterior structures. However, the stage when the ectoderm will develop only into forebrain was not noticed by Mangold and von Woellwarth (1950) who isolated presumptive neural tissue from the mid-gastrula of *Triton*, and its existence can perhaps not yet be taken as certain.

It will be clear from the last few paragraphs that most recent authors consider that at most very few different substances are involved in the regional determination of the neural plate. Dalcq discusses the question in terms of two, one for the prechordal plate and one for the rest of the chorda-mesoderm. Nieuwkoop considers that there are only two factors,

those responsible for the initial forebrain induction and the later transforming action. If the whole regional determination is to be attributed to quantitative variations in so few substances the responsibility for most of the details of the structure of the neural system must be attributed to processes of self-individuation; that is to say, one must suppose that the process of induction gives to a particular region only a general specification to form forebrain or midbrain or some other part, and that the details of the structure of the organ are elaborated by processes going on within the reacting material.

As Lehmann (1948*b*) has pointed out, a self-individuating piece of tissue seems usually to produce a certain range of structures quite completely, and to lack the structures outside that range altogether, rather than to cause the appearance of something which could be considered as a reduced edition of the whole. We have seen an example of this in Nieuwkoop's finding (p. 457) that an induced embryo which contains the trunk will also contain all levels of the brain up to some point at which it stops, the more anterior levels being completely absent. This can be interpreted as indicating, not that there are separate different inducing substances for each level which may or may not be present, but that a given mass of induced neural tissue moulds itself into a neural system which is fully formed as far as it goes and misses out the other parts entirely (Fig. 20.27).

The occurrence of self-individuation in the mesoderm as well as in the neural system has been very strikingly demonstrated by Holtfreter (1943*b*). He cut out the blastopore lip region from a young gastrula of *Triton* and treated it with alkaline solution. The tissue then becomes disaggregated and the cells fall apart. They can be thoroughly stirred round so that their original arrangement is quite lost (as can be demonstrated by mixing together cells from a vitally stained with those from an unstained embryo). The loose cells can then be caused to aggregate again by placing them in acid saline. After re-aggregation is complete the mass can be implanted into the blastocoele of the host embryo; and it is then found to differentiate into coherently arranged tissues and to induce a neural axis which has well-defined regional properties. Thus the re-aggregated organiser cells have produced within themselves a fairly high degree of morphological organisation, clearly by a process of self-individuation.

We have so far considered the interaction between the sheet of inducing mesoderm and the overlying ectoderm as though these two remained the whole time in stationary contact with one another. It is, of course, clear that this is not really the case. The patterns of regional differences within the mesoderm and overlying neural plate are coming into being at a time

when energetic morphogenetic movements are going on; the mesoderm is invaginating at the blastopore and is moving forward under the presumptive neural plate, which at the same time is streaming backwards above it towards the blastopore. Nothing very definite is known about the significance of these movements for the processes of regionalisation, but it seems almost impossible to believe that they are in fact without importance. It seems probable, indeed, that it is to the movements of gastrulation that one should look for an explanation of the field of quantitative variation in inducing capacity which all authors seem to agree must exist within the mesoderm. If an evocating substance begins to be released in the mesoderm at the time this invaginates through the blastopore,

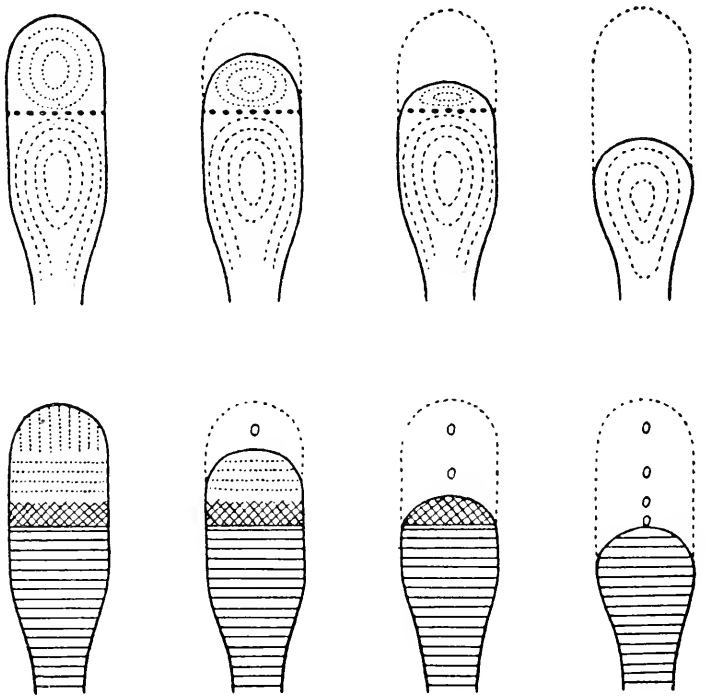


FIGURE 20.27

Diagram to illustrate the results of partial inhibition of the development of the brain. The upper row shows, on the left, the normal situation with two fields, for forebrain and for mid- and hindbrain. On the right of this row are three stages of inhibition; note that the more posterior field is not reduced at all until the anterior one has disappeared entirely. Below are diagrams of the types of brain corresponding to the fields, with indications of the telencephalon, diencephalon, mesencephalon and myelencephalon. (From Lehmann 1948.)

by the late gastrula stage it would presumably have attained a higher concentration in the more anterior mesoderm, which has been invaginated for a longer time, than in the more posterior regions. Similarly one might expect it to spread out laterally from the dorsal midline. Processes of this kind will give rise to a graded field of evocator concentration within the mesoderm (Waddington 1940*a*, Waddington and Yao 1950) (Fig. 20.28).

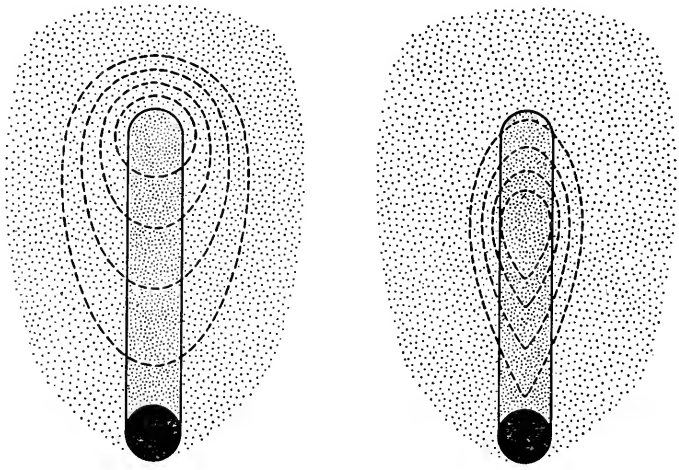


FIGURE 20.28

To illustrate the time relations of organiser action. The drawing on the left represents the archenteron roof in a late gastrula, the black circle being the blastopore. If we suppose that the evocator is liberated in the median strip (close dots), and diffuses laterally, it will have spread into a field represented by the dashed contours. The drawing on the right represents the presumptive neural plate, which has been moving down towards the blastopore, passing over the already invaginated mesoderm. The concentration of evocator which has diffused into it from below will be approximately as indicated by the contours. (From Waddington and Yao 1950.)

As these authors have also pointed out, the movement of invagination will have another consequence in that at any given time the posterior end of the neural plate will have been underlain by mesoderm for a longer period than the anterior end. It may be surmised that the period of time for which the inducing mesoderm acts may have an influence on the regional character of the neural tissue which is produced. Waddington and Deuchar (1952) attempted to demonstrate this. From a late gastrula stage, pieces of presumptive neural plate were removed and transplanted into young gastrula hosts in such a way that they were exposed to the

action of the archenteron roof for a second time. It was hoped that by prolonging the period of induction in this way some change would be made in the regional character of the induced neural tissue, or even that it would be converted into posterior mesoderm which, in normal development, is the fate of that part of the neural plate which is longest underlain. Little effect was, however, produced. Nevertheless it remains true that during the induction of the neural plate we are dealing with a system which involves a considerable amount of relative movement, and it seems most probable that the time-relations both in the production of the evocating substance and in the period of its action on the overlying ectoderm will eventually be found to play some part in the process.

This summary of recent work on the induction of the neural plate, incomplete though it is, has probably sufficed to show how complex a problem is presented by even a comparatively simple instance of individuation. The more one looks into the details of what actually occurs in neural induction the more paradoxical the phenomena appear to be. For instance, we have seen that the neural crest appears to represent a weak grade of induction, yet the first visible sign of the formation of the neural plate occurs not in the dorsal midline but at the margins from which the neural crest will later develop; it is in this region that the cells first assume the elongated columnar shape which will later be characteristic of the whole neural epithelium (cf. Fig. 20.21, p. 451). Why should it be in a region of apparently weak action that an effect is first produced? Again, is it not paradoxical that it is at the anterior end of the neural plate, which is underlain for the shortest time by the archenteron roof, that the neural tube attains its greatest dimensions, while in more posterior regions it is smaller in cross-sectional area, and the very posterior end of the plate, which has been successively underlain by all the levels of the archenteron from anterior to posterior, does not develop into neural tissue at all but forms the mesoderm of the tail? We can certainly not pretend to have any explanation of these facts as yet.

In spite of all the work that has been done on the regional determination of the neural plate, we still find ourselves forced to appeal to the mysterious process of 'self-individuation' to explain the appearance of pattern. This is true both of the pattern in which the different inducing capacities are arranged in the mesoderm sheet, and of the details of the form of the neural organs such as the forebrain, midbrain, etc. By 'self-individuation' we in fact mean no more than that the pattern arises without any ascertainable antecedents. We have seen earlier that both Henke and Turing have discussed ways in which such spontaneous appearance of pattern might be supposed to occur; but it is unsatisfactory to have to rely on mechanisms

of the kind they suggest to account for such complex forms as those of the parts of the brain, and we must certainly continue to search for more definite bases on which the patterns could be built.

SUGGESTED READING

Bonner 1952, Holtfreter 1943–44, Needham 1936*a*, Weiss 1950*b*, Stern 1954.

For an early and still most stimulating account of cell-specific adhesiveness (in sponges) see Huxley 1911, 1921.

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And to make an end is to make a beginning.
The end is where we start from.

★ ★ ★ ★ ★

We shall not cease from exploration
And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.

From *Little Gidding* by T. S. Eliot.

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The titles of some of the more frequently mentioned journals have been contracted as follows:

AB	*Archives de Biologie.*
BR	*Biological Reviews.*
CSHS	*Cold Spring Harbor Symposia on Quantitative Biology.*
G	*Genetics.*
JEB	*Journal of Experimental Biology.*
JEZ	*Journal of Experimental Zoology.*
JEEM	*Journal of Embryology and Experimental Morphology.*
JG	*Journal of Genetics.*
N	*Nature*, London.
PNAS	*Proceedings of the National Academy of Science*, Washington.
PRSE,B	*Proceedings of the Royal Society of Edinburgh*, series B.
PRSL,B	*Proceedings of the Royal Society of London*, series B.
PTRSL,B	*Philosophical Transactions of the Royal Society of London*, series B.
PR	*Physiological Reviews.*
PZ	*Physiological Zoology.*
RA	*Roux' Archiv für Entwicklungsmechanik der Organismen.*
RSZ	*Revue Suisse de Zoologie.*
S	*Science.*
SYMSEB	*Symposia of the Society for Experimental Biology.*
ZIAV	*Zeitschrift für induktive Abstammungs- und Vererbungslehre.*

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AUTHOR INDEX

References to lengthy discussions, or to contexts in which the same author is referred to several times on contiguous pages, are followed by the letter 's', e.g. 142s.

SUBJECT INDEX

When a page-reference denotes the beginning of a lengthy discussion, it is followed by the letter 's', e.g. 124s. For certain common technical words, references are given only to a few key places which can serve as a definition.