PRINCIPLES OF EMBRYOLOGY

BY

C. H. WADDINGTON

SC.D., F.R.S.

Professor of Animal Genetics
University of Edinburgh

WITH 186 FIGURES

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.

CONTENTS

PART TWO

THE FUNDAMENTAL MECHANISMS OF DEVELOPMENT

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 lifetimes. 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 lamentable 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

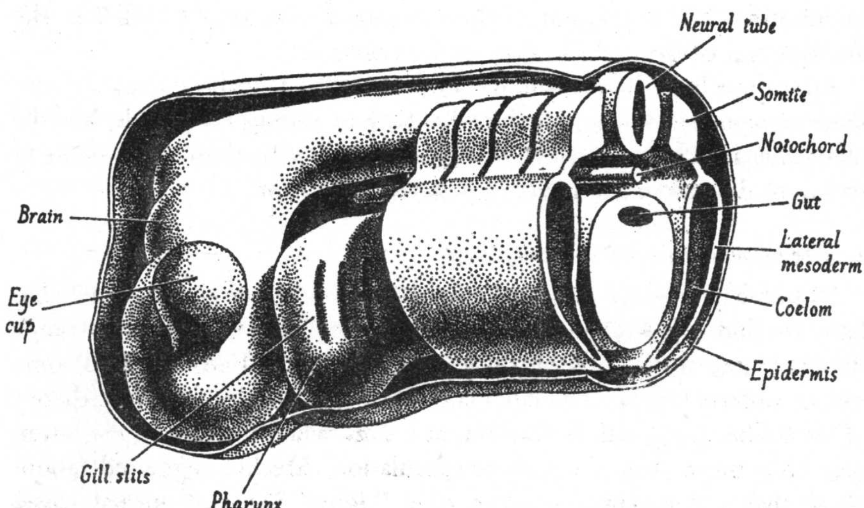

FIGURE 1.1

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-