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The Elements of Experimental Embryology

THE ELEMENTS OF EXPERIMENTAL EMBRYOLOGY

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THE ELEMENTS OF EXPERIMENTAL EMBRYOLOGY

To

ROSS HARRISON and HANS SPEMANN

PREFACE

A few words are needed to explain the scope of this book. The study of the developmental processes of animals is an enormous field, of which only a small fraction can be dealt with in a volume of this size. The observational and comparative study of embryology falls outside the boundaries of this series; in any case, it has already been treated in numerous authoritative works. Even on the experimental and physiological side, however, there remains the difficulty of selection from the vast mass of somewhat heterogeneous material which many lines of research have provided for consideration and synthesis.

In the first place, development is not merely an affair of early stages; it continues, though usually at a diminishing rate, throughout life. The processes of amphibian metamorphosis or of human puberty; the form-changes accompanying growth; senescence and natural death itself—these are all aspects of development; and so, of course, is regeneration.

We feel that it is impossible to treat the whole life-cycle in a single volume, and have accordingly set an arbitrary limit to our material. We have deliberately restricted ourselves to the early period of development, from the undifferentiated condition up to the stage at which the main organs are laid down and their tissues histologically differentiated—in other words, to Wilhelm Roux's "prefunctional period". Growth, absolute and relative; the effects of function on structure and on size; the morphogenetic effects of hormones—the details of these and of other related topics we have deliberately omitted, and we have contented ourselves with the addition of a final chapter in which the main peculiarities of the functional period are contrasted with those of the pre-functional period of primary differentiation. Any satisfactory treatment of the latter portion of the developmental cycle would require a separate volume.

In the second place, within the period of early development, we have exercised a further selection. In a new field of biology such as this, there are always two levels of approach. One of these is broadly biological, while the other is physiological in the stricter sense. The prime aim of the worker approaching the problem on the physiological level will always be to analyse the processes involved in terms of physics and chemistry. The worker on the biological level will aim at discovering general rules and laws which he is content to leave to his physiological colleague for future analysis in more fundamental terms, but which, meanwhile, will give coherence and a first degree of scientific explanation to his facts. Both methods are necessary for progress; and while most biologists hope and expect that one day their laws will, thanks to the labours of their physiological colleagues, be made comprehensible in the most fundamental physico-chemical terms, they can reflect that it is they who must first reveal the existence of these laws before the pure physiologist can hope to begin his analysis. The biologist can also remember that these laws have their own validity on their own level, whether they be physico-chemically analysed or not.

We may take a salient example from the contents of this book. Spemann's discovery of "organisers" in the process of gastrulation of Amphibia, and the extension of the concept to other stages of development and to other groups of organisms, have made it possible to understand on the biological level many processes of development which were previously obscure. At the moment we can only throw out crude guesses as to the underlying physiology of organisers and their effects, but the discovery opens a new field of research to physiologists, which they themselves would not have been likely to hit upon for many years. And even if and when the physiological analysis has been made, the empirical biological laws concerning organisers will not lose their validity or their interest; they will merely have been extended and deepened.

At the present moment, research into developmental problems is being actively prosecuted on both the biological and the physiological levels. Following up the early work of Roux, Hertwig, Driesch, Herbst, Jenkinson, Delage, Brachet, Morgan, and Wilson, a flourishing school of *Entwicklungsmechanik* has grown up in Germany, and another, no less successful, in the United States. Meanwhile, on the physiological side, the advance has also been striking, and we may perhaps cite as particular examples such works as Fauré-Fremiet's *Cinétique du Développement*; Gray's *Experimental Cytology*; Dalcq's *Bases Physiologiques de la Fécondation*; and Needham's classic book on *Chemical Embryology*.

So far, however, little progress has been made in equating the results of the two lines of approach, and it seems clear that a considerable time must elapse before it will be possible to do so satisfactorily. At the moment the two fields are almost as unrelated as were, through most of the nineteenth century, the cytological and the experimental-genetic approaches to the problem of heredity, which are now inseparable.

That being so, we have not attempted to include the results of the purely physiological study of development in this survey. This means that we have deliberately excluded such topics as the physiology of fertilisation, the mechanics of cleavage, and the biochemistry of the egg and embryo, save where they have a specific bearing on the biological problems involved.

In other words, what we have attempted to do is to give some account of the results of the experimental attack on the problem of the biology of differentiation—the production of an organised whole with differentiated parts out of an entirely or relatively undifferentiated portion of living material. Almost the only short books on this subject since Jenkinson's *Experimental Embryology* and his (posthumous) *Lectures* are Brachet's *L'Œuf et les Facteurs de l'Ontogénèse*, Dürken's *Grundriss der Entwicklungsmechanik*, Weiss' *Entwicklungsphysiologie der Tiere*, and de Beer's *Introduction to Experimental Embryology*; and each of these treats the subject along rather different lines. Among larger works, Wilson's *The Cell*, Morgan's *Experimental Embryology*, Dürken's *Lehrbuch der Experimentalzoologie*, and Schleip's *Determination der Primitiventwicklung* are the most important which have appeared since the pioneer works on the subject. A perusal of them will suffice to show the extreme diversity of their lines of approach. What we have felt is that at present there exists in the subject a vast body of facts and a relative paucity of general principles. We have accordingly aimed at marshalling the facts under the banner of general principles wherever possible, even when the principle seemed to be only provisional.

Many of the illustrations have been drawn specially for this book by Miss B. Phillipson, to whose care and skill we wish here to make acknowledgments. Particular thanks are due to Miss P. Coombs for her help with typing and many other details of preparing the book for press. Acknowledgment is hereby made to those authors and publishers of the journals whose names appear in the legends to the figures, by whose courtesy they are here reproduced.

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In conclusion, we should like to acknowledge our debt to the late Dr J. W. Jenkinson, an Oxford man, and the pioneer of Experimental Embryology in this country, and to express our deep appreciation of the care and skill which the Cambridge University Press has expended on the production of this volume.

J. S. H.
G. R. DE B.

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Chapter I

HISTORICAL INTRODUCTION TO THE PROBLEM OF DIFFERENTIATION

§ 1

The production of the adult living organism with all its complexity out of a simple egg (or its equivalent in the terminology of the ancients) is a phenomenon and a problem which has attracted the attention of philosophers as well as of scientists for over two thousand years. To give a brief account of the history of ideas relating to this problem is no easy matter, but the task is fortunately facilitated by the fact that Dr E. S. Russell and Prof. F. J. Cole, F.R.S. have recently devoted volumes to certain aspects of this subject, and to the reader who desires to become better acquainted with it, no better advice can be given than to refer him to *The Interpretation of Development and Heredity*, and to *Early Theories of Sexual Generation*. The historical section of Dr Needham's *Chemical Embryology*, and various works of Dr Charles Singer also provide much valuable information.

Meanwhile, a brief attempt will be made in the following few pages to outline the essential features of the chief schools of thought concerning problems of development, in order to show how the modern science of experimental embryology came into being, and to present it in its proper historical setting.

The kernel of the problem is the appearance during individual development of complexity of form and of function where previously no such complexity existed. In the past, there have existed two sharply contrasted sets of theories to account for it. One view accepts the phenomenon as essentially a genesis of diversity, a new creation, and attempts to understand it as such. This coming into existence of new complexity of form and function during development is styled *epigenesis*.

The difficulties which other thinkers experienced in trying to understand how epigenesis may be brought about led them to deny that it exists: i.e. to say that there is no fresh creation of diversity in development from the egg, but only a realisation, expansion, and rendering visible of a pre-existing diversity. *Preformation* is the fundamental assumption of views of this type, and they are classed together as preformationist theories. But the doctrine of preformation, however, met with even graver obstacles, both logical and empirical, than the opposite view, and biological opinion is now united in maintaining the existence of a true epigenesis in development. In recent years, however, the discoveries of genetics have reintroduced certain elements of the preformationist theory, but in more subtle form. As will be seen later, the modern view is rigorously preformationist as regards the hereditary constitution of an organism, but rigorously epigenetic as regards its embryological development.

To a large extent, the preformationist view assumes as already given that which the epigenetic attempts to study and to explain; and the problem is complicated by the fact that notions of embryonic development have been confused with concepts of heredity. This is evident in the attempt, on the part of the author of *Peri Gones* in the Hippocratic corpus, to explain development by assuming a part-to-part correspondence between the parts of the body of the parent and those of the offspring: the corresponding parts being related to one another via the "semen", or, as would now be said, via the germ-cells. By assuming that the embryo at its earliest stage is a minute replica of the adult, its parts having been "preformed" by representative particles coming from the corresponding parts of the parent, the preformationist hypothesis attempts to solve at one stroke both the problem of hereditary resemblance between generations and the problem of development within each generation.

This view was in reality shattered by Aristotle's criticism, but it was revived and widely held during the seventeenth and eighteenth centuries, largely owing to the fact that mechanistic explanations had come into vogue, and it seemed impossible to understand epigenesis on mechanistic lines. One of the foremost exponents of the preformationist hypothesis was Charles Bonnet. His views were freed from the crude idea that the preformation in the egg was spatially identical with the arrangement of parts in the adult and fully developed animal, or that the "homunculus" in the sperm, with the head, trunk, arms and legs which it was supposed to have (and which certain over-enthusiastic observers claimed to have seen through their microscopes; see Cole's *Early Theories of Sexual Generation*) only required to increase in size, as if inflated by a pump, in order to produce development. Instead of regarding the rudiments of the organs as being preformed in their definitive adult positions, Bonnet imagined them as "organic points" which subsequently had to undergo considerable translocation and rearrangement. He was thus able to reconcile his belief in preformation with the empirical fact that the germ or blastoderm of the early chick showed no resemblance to a hen.

Bonnet's theories were ahead of his facts, and, indeed, he seems to have been proud of it, for he refers to the preformationist view as "the most striking victory of reason over the senses". The hypothesis of such an invisible and elastic preformation was perhaps permissible in Bonnet's day, but later observational and experimental evidence has rendered it utterly untenable. Further, a rigid preformationist view which asserts that the egg is a miniature and preformed adult, necessarily implies that the egg must also contain the eggs for the next generation; the latter eggs must therefore also contain miniature embryos and the eggs for their subsequent generations. Bonnet realised that an *emboîtement* or encasement of this kind *ad infinitum* would be an absurdity. (Incidentally, it may be noticed that if it were true, phylogenetic evolution—unless it too were preformed and predetermined—would be an impossibility.) But then, if *all* subsequent generations are not preformed in miniature now, there must come a time when they *are* determined and preformed. Before this time they were neither determined nor preformed, and this making of a new determination, albeit pushed into the future, is the antithesis of preformation.

If pushed to its extreme conception of infinite encasement, then preformation is absurd; if not pushed to this extreme, preformation will not account for the determination of ultimate future generations; and if it did apply, preformation would be an unsatisfactory view in that it assumes that the diversity which is progressively manifested in development is ready-made at the start, and in no way attempts to explain it causally or to interpret it in simpler terms.

§ 2

Logically, the preformationist view is associated with the notion of separate particles being transmitted from parent to offspring, though the converse does not hold. In preformationist theory, the hypothetical particles establish the one-to-one link between the corresponding organs and parts of parent and offspring, whereas the modern view, which combines an epigenetic outlook on development with the particulate theories of neo-Mendelism, denies any such simple correspondence between hereditary germinal unit and developed adult character. Darwin's theory of pangenesis resembles that of the Hippocratic writer in this respect, the pangens being supposed to come from all parts of the body of the parent and to be transmitted, via the germ-cells or "semen", to the offspring whose development they mould. Embryologically, however, Darwin's theory is vague, and leaves the question of preformation open. Weismann's theory of the germ-plasm, in which the *determinants* are regarded as representing the predetermined but not spatially preformed diversity of the future embryo, differs from that of previous preformationists in that the particles are regarded as coming, *not* from the corresponding parts of the body of the parent, but from the germ-plasm, of which each generation of individual organisms is held to be nothing but the life-custodian. Weismann identified the determinants with the material in the nuclei of the cells, which material he (wrongly) supposed was divided unequally in the process of division or cleavage of the egg, so as to form a mosaic, the pieces (cells or regions) of which would then contain different determinants and would therefore be predetermined to develop in their respective different and definite directions.

According to the writer of the Hippocratic treatise *Peri Gones* and to Darwin, therefore, offspring resembles parent because the particles responsible for the development of the parts of the offspring come from the corresponding parts of the parent. According to Weismann, however, offspring resembles parent because both have derived similar particles (determinants) from a common source—the germ-plasm.

The question of the *origin* of the particles or hereditary factors and of their *distribution* from the parent to the offspring is one which principally concerns the science of genetics. The modern tendency is to accept the principle of a germ-plasm while recognising that it is not as inaccessible to the modifying action of external factors as Weismann contended. The question of the *function* of the particles or factors in converting the fertilised egg into the body of the adult is the concern of that modern and rather special branch of embryology usually called physiological genetics.

Before dealing with the conclusion derived directly from experimental work, a moment's attention may be turned to philosophical criticisms of the preformationist view that particles, determinants, or any hereditarily transmitted units or factors, can "explain" development. First of all, Aristotle pointed out that certain features in which offspring resembled parent could not be ascribed to the transmission of particles from corresponding parts, for the latter might be dead structures like nails or hair from which no particles could be expected to come, or again they might be such characters as timbre of voice or method of gait. He goes on to say, by way of illustration, that if a son resembles his father, the shoes he wears will be like his father's shoes, yet there can, of course, be no question of particles here. In other cases, resemblance may refer to structure, plan or configuration rather than to the material of which it is composed, and it is hard to see how particles can represent such structure, plan or configuration. Again, how is the eventual beard of a son to be explained if he was born to a beardless father? To these objections might be added the insuperable difficulty of accounting for the production of offspring structurally different from the parent, as when the egg laid by a queen bee develops into a worker, or, even more generally, when a mother bears a son or a man fathers a daughter.

If, then, particles coming from corresponding parts are not required in some cases and cannot be resorted to in others in order to explain development and hereditary resemblance, why should they be postulated in any case? This, of course, concerns genetics as much as embryology, but Aristotle came very close to the crucial problem of the latter when he wrote: "either all the parts, as heart, lung, liver, eye, and all the rest, come into being together, or in succession....That the former is not the fact is plain even to the senses, for some of the parts are clearly visible as already existing in the embryo while others are not; that it is not because of their being too small that they are not visible is clear, for the lung is of greater size than the heart, and yet appears later than the heart in the original development".[1]

Simple observation, therefore, had even in Aristotle's time given the lie direct to the view that the embryo is a spatially preformed miniature adult. Similar but more exhaustive and more crucial observational evidence against the preformationist view was supplied by William Harvey (who referred to development as "*epigenesin sive partium superadditionem*") and, notably, by Caspar Friedrich Wolff. The conclusion to which the latter came is the same as that of Aristotle. In the earliest stages of the development of the fowl, the microscope reveals the presence of little globules heaped together without coherence, and a miniature of the adult simply does not exist. Further, no refuge can be taken in the assumption that the miniature is too small to be seen, for its parts (globules) are clearly visible, and, *a fortiori*, therefore, the whole. The plain fact is that the miniature of the adult is not there.

The necessary epigenetic correlate of this fact has been admirably put by Delage in the following words: "latent or potential characters are absent characters.... The egg contains nothing beyond the special physico-chemical constitution that confers upon it its individual properties *qua* cell. It is evident that this constitution is the condition of future characters, but this condition is in the egg extremely incomplete, and to say that it is complete but latent is to falsify the state of affairs. What is lacking to complete the conditions does not exist in the egg in a state of inhibition, but outside the egg altogether, and can equally well occur or not occur at the required moment. Ontogeny is *not* completely determined in the egg".[1] We might sum up the position by saying that to maintain the full preformationist view would partake of the nature of fraudulent book-keeping.

There is no way of saving the view that the adult is preformed in the egg as a diminutive replica. The more subtle idea of Bonnet's, of preformed "organic points", or of determinants unequally distributed between the cells into which the egg divides, also met its doom a century ago, when Etienne Geoffroy St Hilaire (1826) experi-

[1] Quoted from Russell, *loc. cit.*