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ORGANISERS AND GENES

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by

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PREFACE

This short discussion is not intended as a general survey of the whole field of experimental embryology. It is an account of the lines of thought suggested by a single set of phenomena, those of embryonic induction. I have attempted to approach this particular aspect of development from as many angles as possible; and it is fortunate that the process of induction is not only one which lies open to causal analysis, but is also one which impinges on nearly all the fundamental problems of development. It is naturally important to enquire how generally one can apply the principles derived from the study of so important a phenomenon. I have not, however, attempted any extended discussion of the relation between the inductive mechanisms of vertebrates and the causal processes which have been discovered in embryological investigations of invertebrates. I have instead devoted some space to pointing out the similarities between the concepts derived from the consideration of the organiser and those which arise in connection with the developmental effects of genes. The two sets of phenomena have of course been investigated in different organisms, but the principles of genetics are so uniform throughout the animal kingdom that it may not be too much to hope that processes occurring in one group may provide a valuable guide to those in another.

It should perhaps be pointed out that throughout the discussion I have tried to adopt an inductive approach. Thus in discussing induction we find evidence that there are two aspects to the matter, which have been called evocation and individuation. It will avoid confusion if it is remembered that these are essentially names for two subjects of investigation rather than for two ultimately different principles of explanation. In fact, the later discussion shows that some at least of the problems of individuation can probably be solved in the same terms as those of evocation.

It is a consequence of the same method of approach that the discussion of the most abstract concepts, such as fields and organisation, comes at the end of the book rather than at the beginning.

I should like to thank G. Bateson and Dr J. Holtfreter, whe have read several of the chapters and discussed them with me; and I am also grateful to John Piper for his interpretation of my somewhat romantic conceit, the epigenetic landscape. My greatest obligation, however, is to Dr Joseph Needham, who has been my close collaborator during most of this book's gestation.

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THE CAUSAL ANALYSIS OF
DEVELOPMENT

THE diversity of living beings is much greater than that of the
non-living things with which man comes in contact. Biology
therefore remained a mainly descriptive science long after physics
and chemistry had begun to analyse the causal connections
between happenings in the inorganic world. But gradually
biology too became predominantly a causal discipline, and the
section of it which forms the subject of this book will be dis-
cussed entirely with the object of deepening our knowledge of
the essential interrelations which make up its causal network.

When biology is regarded from this point of view, two sets of
phenomena tend at first to become separated from one another.
A living being functions from day to day by a large number of
comparatively rapid processes: digestion, respiration, muscular
contractions, nervous impulses and so on. These are all intimately
related to one another, and can easily be taken as a single field
of study, the investigation of the organism as a going concern.
But organisms are also parts of connected chains of events of much
longer duration. An individual begins life as a fertilised egg,
develops, and dies; the individuals are connected one with another
by the processes of biological inheritance; and whole series of
individuals, which may be grouped into larger units such as
species and genera, are involved in the long-range process of
evolution. These three types of change form the subject-matter of
the sciences of embryology, genetics and evolution, which again
form a group whose interconnections are obvious and unavoidable.
Undoubtedly both the long- and the short-range biological changes
should be subsumed within a single general biological theory.
But in practice biology is not yet in a position to do this effectively.
We can only look with envy at physicists who can bring within
one consistent framework the behaviour of electrons, infinitesimal
in space and fleeting in time, and the vast and ponderous evo-
lution of stellar systems. While we keep in mind the necessity of
working towards such a synthetic scheme as a final goal, we only

too often find ourselves forced to accept the natural separation of the biological disciplines into the two great groups we have mentioned; the synchronic or physiological, and the diachronic or developmental; or the cyclic processes, repeatable in one life-history, and the progressive, which an individual undergoes only once. Bridges between these two fields already exist, though in somewhat skeleton form; the study of the chemical nature of the gene, and its mode of action, as well as the physiological and biochemical study of development, are beginning to bring the concepts of the two types of biology into contact.[1]

In this book I shall not be primarily concerned to discuss the relations between synchronic and diachronic biology, although some aspects of this question will be touched on. My first aim, however, is to contribute to the bridging of a narrower gap, which exists between the two fields of embryology and genetics within the general sphere of diachronic biology.

The causal analysis of development on the whole lags behind that of the day-to-day functioning of organisms. It is true that the elements of the theory of development on the largest time-scale, that is to say, the theory of evolution, was one of the earliest biological theories to be enunciated in a satisfactory form; but its elaboration has been very slow, and only in the last few years have the theoretical researches of Wright, Fisher, Haldane and Darling-ton, and the practical work of Tchetverikov, Sturtevant, Dobzhan-sky and others, carried our understanding much beyond the point reached by Darwin. On the intermediate time-scale of genetics, much more progress has been made, and this is one of the most fully developed of all biological sciences. But in the short end of the range, again, the causal study of development has also ad-vanced only very slowly.

The older investigations of embryonic development led to results of two different kinds, which were not only apparently diametrically opposed to one another, but were each of them such that they offered no obvious hope for further insight into the processes involved. On the one hand, it was found that, in many eggs, each part was capable of forming a certain part of the adult, and that part only. The egg was a mosaic of regions, each with a definite potency for development. No causal mechanism could be discovered; the eggs just developed, and the parts just developed;

[1] Waddington, 1939 a.

and that was all that could be found out. On the other hand, eggs of certain other species showed the contrary behaviour; any part of the egg could, it seemed (though this is now considered very doubtful), become a whole embryo. Again, no causal mechanism appeared; and in fact some biologists, such as Driesch, gave up hope of discovering a material causal mechanism which could explain the facts.

Attacking the problem indirectly through a study of heredity, genetics in the early years of the century did indeed succeed in revealing some true causal antecedents of adult structures and functions. The method could, by its very nature, only show that genes are responsible for the development of characters which may be different in two organisms which can nevertheless breed together. These characters are all dependent on nuclear factors; the properties of the egg cytoplasm are not susceptible of analysis by the same method. Even if we assume, as we are probably justified in doing, that these cytoplasmic properties are themselves dependent on the genetic endowment of the mother by whose body the eggs are formed, that assumption, important though it may be in connection with the theory of evolution, is not relevant when we are considering the mechanism of development during a single individual life. Moreover, the discovery of genetic factors reveals only the first link of a chain of causal events, whose other end, the adult character, is known, but whose intermediate links require elucidation. The genes cannot be regarded as immediately effective in causing the successive processes of differentiation, although they are undoubtedly the fundamental elements which ultimately control them. A coherent theory of development cannot be founded on the known properties of genes; in fact, it seems much more hopeful to try to fit our somewhat scanty knowledge of the developmental actions of genes into a framework founded in the first instance on the direct experimental study of development.

The essential question for a theory of development is this: What is the immediate causal network underlying this particular process of differentiation occurring at this particular time? The first partial answer to such a question was given by Spemann.[1] His success was partly due to the elaboration of an adequate technique of operation. But it was also dependent on the clear formulation

[1] Spemann, 1918; Spemann & Mangold, 1924.

of the essential query just mentioned. It was known that in the amphibian gastrula the dorsal half of the animal hemisphere develops into neural tissue, the ventral half into epidermis. By a series of experiments which have become classical, Spemann showed that the reason for this difference in behaviour lies in the fact that the dorsal region comes in contact with the tissues which are invaginated to become mesoderm, and that the neural differentiation is a response to a stimulus emanating from the mesoderm.

The principle that the differentiation of a certain tissue or organ may be induced by a stimulus exerted by some other part of the egg had been adumbrated by Roux in his idea of dependent differentiation, and was partly confirmed by Spemann's earlier researches on the interaction between the eye-cup and lens. But it was the work on the neural plate, which is the first-formed and primary organ of the vertebrate body, which demonstrated the importance and scope of the mechanism. The causal analysis of development may be said to have first started with this discovery. At the same time, it is obvious that we have only a beginning of an answer to our essential query. No "stimulus", nor single cause, is itself an adequate explanation of anything. We must hope eventually to know the whole complex system of actions and interactions which constitute the differentiation.

The significance, and at the same time the crudity, of the ideas involved in Spemann's discovery may perhaps best be appreciated by an analogy. It has been known for some hundreds of years that the "cause" of muscular contraction is stimulation by a nerve. The statement that the "cause" of an embryonic differentiation is stimulation by an organiser is just as basic, in its own sphere, and just as crude. Both statements obviously require, and at the same time provide a guide for, further investigations into the nature of the stimulus and the nature and mechanism of the response.

ORGANISERS IN DIFFERENT CLASSES
OF VERTEBRATES

BEFORE entering on the discussion of the mode of action of
organisers, it seems advisable to summarise shortly what is known
about the organisers of the various groups of vertebrates.

Amphibia.

The main facts about the positions and functions of the am-
phibian organisers, on which Spemann[1] did his classical work, are
well known and have recently been reviewed in several publi-
cations. The first to become active during development is known as
the primary organiser, and lies anterior and dorsal to the blasto-
pore in the early gastrula; it extends laterally for a considerable
distance on each side of the dorsal midline; in fact, Holtfreter[2] has
recently shown that in some respects even the most ventral tissue
of the marginal zone must be considered to belong to it.

The activity of the organiser is most conclusively demonstrated
by grafting experiments. On being inserted into a new region of
the gastrula, a fragment of tissue from the primary organiser
pursues its own characteristic development, invaginating into the
interior of the embryo and developing into mesodermal tissues
such as notochord and somites. Some slight change of develop-
mental fate may be involved in this differentiation (p. 100) and
part of the organiser tissue may develop atypically into neural
tissue. A much more profound change in developmental fate is,
however, produced in the neighbouring ectoderm; even if the
graft has been made into a region where the ectoderm would
normally develop into epidermis, the organiser stimulus causes it
to differentiate into neural tissue. The induced neural tissue and
the mesodermal structures from the graft often become adjusted
to one another so as to form a comparatively normal embryonic
axis; and a similar inducing action on the endoderm may provide
this embryonic rudiment with an appropriate gut.

[1] Spemann, 1938.   [2] Holtfreter, 1936, 1938a.

The primary organiser is succeeded by a series of others, which may be spoken of as secondary and tertiary organisers. Of these perhaps the best known example is the eye-cup, which induces the formation of the lens from the ectoderm with which it comes in contact. The other placodes, of the ear and nose, for example, are also dependent on inducing stimuli, which may in some cases proceed simultaneously from several different organiser regions; Holtfreter[1] has spoken of the ear as being formed under a cross-fire of organisers. Similarly, induction phenomena have been demonstrated in the development of many of the later formed embryonic organs, such as the mouth, in the formation of organs during metamorphosis and probably in regeneration in the adult.
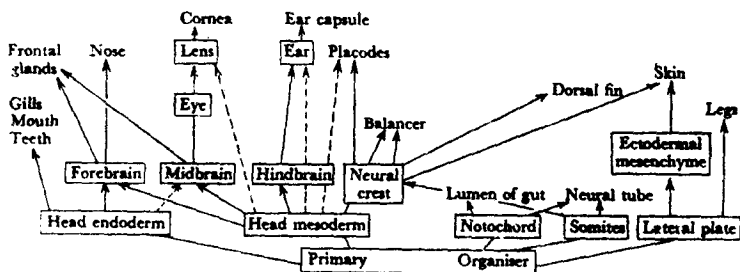

Fig. 1. Organisers in the Amphibia. The main organiser regions are enclosed in rectangles. The lines join organs which are developed one from the other, while the arrows show inducing relations, the dotted ones being of less importance. (After Holtfreter.)

Holtfreter[2] has recently summarised our knowledge of the succession of primary, secondary, tertiary, etc. organisers in Amphibia in a diagram which is reproduced, with some minor alterations, in Fig. 1. In this diagram, the most important inducing influences are indicated by full arrows, less powerful ones by dotted arrows; developmental forms of the same mass of tissue (e.g. the eye which is formed out of the midbrain) are connected by a line without an arrow.

Fish.

The gastrula of cyclostomes is extremely similar in general conformation to that of the newt. Weissenberg[3] has given an exact description of the location of the presumptive regions of the lamprey and shown that their arrangement follows very closely

[1] Holtfreter, 1935 a.   [2] Holtfreter, 1938 a.   [3] Weissenberg, 1934.

those described by Vogt in *Triton*, the main difference being that in the cyclostomes the presumptive mesoderm does not extend completely round the gastrula, but is absent on the ventral side. Bytinski-Salz[1] has repeated many of the classical amphibian experiments with this material, and succeeded in proving that here also the organiser is located just dorsal to the blastopore.

In the meroblastic eggs of teleosts, the conditions for gastrulation are of course very unlike those in the Amphibia or Cyclostomes, and the distribution of presumptive areas is modified accordingly.[2] Workers in America and Germany[3] simultaneously succeeded in overcoming the considerable technical difficulties of the material, and discovered that here again the blastopore is not only the centre of gastrulation movements but also the site of an inductive agency. The direct demonstration of the organiser was made by grafting the invaginating meso-endoderm under a new region of the blastodisc; the graft continued its normal development into chorda, somites and gut endoderm, and at the same time induced the formation of a neural plate by the overlying ectoderm. Some of the inductions obtained are extremely complete, except for slight deficiencies in the head region.

Birds.

After their discovery in Amphibia, organisers were next detected in birds.[4] The phenomena in this group are probably not so widely known as those in Amphibia, and have not been recently summarised, and therefore require slightly fuller treatment.

The organisation centre in the Amphibia and fish is situated at the focus of the gastrulation movements by which the endoderm and mesoderm are brought to their final positions under the ectoderm. In the chick, these movements take place in two phases; the endoderm is formed at about the time of laying, while the mesoderm is not formed till the primitive streak stage some hours later. The exact mechanisms involved in these processes are still under dispute, and until certainty has been reached about them, some doubt must remain as to the positions of the presumptive endoderm and mesoderm before the invagination occurs. Until recently the standard account of endoderm formation was that of Patterson, who stated that, in the pigeon, the posterior edge of

[1] Bytinski-Salz, 1937 *a*, *b*; Yamada, 1938 *a*.
[2] Pasteels, 1936; Oppenheimer, 1936 *a*.
[3] Luther, 1935; Oppenheimer, 1934 *a*, *b*, 1936 *b*.   [4] Waddington, 1930.

the blastodisc turns underneath and grows forward as the lower layer. The careful investigations of Jacobson[1] have, however, shown that the endoderm is formed from a region just anterior to the posterior edge. Thus the presumptive endoderm, before its invagination, lies in the posterior part of the circular blastodisc, but its exact extent is not known.

The mesoderm is formed from the primitive streak, which appears as a thickened ridge in the posterior part of the blastoderm shortly after endoderm formation has been completed. The mesoderm, during its formation, is carried from the upper layer (epiblast) into the space between the epiblast and the endoderm; it can therefore be said to be invaginated.

In the stage with a fully formed streak, which is the stage with which the experimental work has been mainly concerned, there is general agreement that the entire axial structures of the embryo (notochord, somites, neural tube) are concentrated round the anterior two-thirds of the streak, with the mesoderm centrally placed. The most peripheral of the definitive mesoderm is invaginated first through the primitive streak and is therefore most centrally placed, while the last-invaginated material, the notochord, is on the lateral boundaries of the mesoderm arc. Outside the arc of mesoderm is the presumptive neural material, also in the form of an arc.

Speculations as to the position of the organisation centre of the chick embryo were made in the early days of Spemann's discovery. Gräper argued that in the chick it is the formation of the endoderm which must be regarded as homologous with invagination in the Amphibia and that one would therefore expect the organisation centre to be located at the posterior edge of the unincubated blastodisc. Wetzel,[3] on the other hand, regarded the primitive streak, in particular its anterior end or Hensen's node, as the true homologue of the blastopore, and at one time suggested that it was the organisation centre. A decision between these possibilities, or even a demonstration that either of them is true, awaited the discovery of a technique of operating on the bird embryo.

The earliest technique to be employed was that of chorio-allantoic grafting; in this method fragments of tissue are isolated

[1] Jacobson, 1938.
[2] Gräper, 1929; Jacobson, 1938; Pasteels, 1937a: Wetzel, 1929a.
[3] Wetzel, 1924; cf. 1929b.

on the highly vascular chorio-allantois of older embryos, where they become invaded by blood vessels which supply them with oxygen and nourishment. The method should allow one to study the capacity for independent differentiation of isolated fragments, but it is open to many criticisms, both on the score of its theoretical validity and of the way in which it has been actually used.[1] The theoretical criticism is that the circulating blood contains a substance capable of inducing neural tissue in *Triton*, and probably in the chick; one cannot therefore expect it to be a "neutral" situation, and the fact that it is not one is shown by the incomplete development of whole blastoderms when isolated upon it. The practical restriction on the use of the method lies in the difficulty of isolating suitable fragments of tissue. All authors using the method, until recently, have isolated fragments containing, as well as ectoderm, either endoderm or mesoderm or both, that is to say, containing inducing tissues. Thus in the early days of such investigations, Hoadley[2] obtained neural differentiation from isolated fragments from pre-primitive streak stages and drew the unjustified conclusion that the determination of neural tissue had already occurred.

Recently Rudnick[3] has obtained neural tissue from fragments of blastoderm which were isolated from endoderm, and dissected before the invagination of mesoderm in such a way that they should not have included any presumptive mesodermal tissues. Abercrombie (unpublished) has evidence to the same effect. The development of neural tissue in these fragments may indicate a real precocious tendency for this type of differentiation, present before and independently of any action of the mesoderm organiser. Such a tendency has frequently been invoked in the Amphibia, but the possibility of it was finally banished by Holtfreter's[4] exogastrulation experiments. In chick material on which the endoderm organiser has already been acting, a similar tendency would perhaps not be too unexpected.

Hoadley was also able to draw an entirely correct conclusion, namely that Hensen's node, which had not been included in some of his grafts, is not essential to the formation of the embryo. This result was confirmed by Wetzel,[5] who obtained differentiation of

[1] Waddington, 1935 a. [2] Hoadley, 1926, 1927.
[3] Rudnick, 1938 b. [4] Holtfreter, 1933 c.
[5] Wetzel, 1929 b.

neural tissue from the posterior parts of blastoderms which he sectioned *in ovo*. The difficulties offered to exact work by the shell and albumen, made it impossible to proceed much-farther with this technique. However, the technique of tissue culture had by this time reached a stage at which it was immediately possible to explant young blastoderms, removed from the egg, and keep them alive in culture long enough for the development of the main axial organs. The explanted embryos are easily operated upon.

The position of endoderm invagination cannot be determined by inspection of the living blastoderm until endoderm formation is nearly complete and the primitive streak is beginning to appear. At this stage the endoderm may be removed and replaced in a different position. The orientation of the endoderm is found to have an influence on the direction in which the primitive streak grows; the streak always tends to elongate towards the region under which lies the anterior end of the endoderm[1]. This pointed to an inducing action of the endoderm, which was subsequently demonstrated; if the endoderm is reversed, so that its anterior end lies under the primitive streak, a new primitive streak may be induced above its posterior end, so that two embryos are developed on the blastoderm, pointing in opposite directions.[2] Dalton[3] argues that this inducing action of the endoderm is normally active even in young primitive streak stages, since he found no differentiation of structures characteristic of the axial mesoderm in chorio-allantoic grafts of the posterior parts of young primitive streaks from which the endoderm had been removed, but this result is rendered somewhat uncertain by the fact that Dalton judged the presence of axial mesoderm only by the presence of its specialised derivatives such as mesonephros. Twiesselmann[4] reports the production of double monsters, apparently by splitting of an organisation centre, when electrolytic injuries are made slightly anterior to the posterior edge of the unincubated blastoderm; it is possible that his injuries affected the endoderm organiser, although he himself suggests that they affected the future primitive streak material. Butler[5] grafted fragments of the unincubated blastoderm on to the chorio-allantois and obtained satisfactory development only from those pieces which included

[1] Waddington, 1930, 1932.   [2] Waddington, 1933 *b*.
[3] Dalton, 1935.   [4] Twiesselmann, 1938.
[5] Butler, 1935.

the posterior segment of the blastoderm, which may be taken as supporting the suggestion that there is an organisation centre in this region.

There is thus good evidence that, as Gräper suggested, the formation of endoderm is closely connected with organisation phenomena. The structure which arises in response to the organising stimulus is the primitive streak, and from the streak a new organising stimulus is exerted on the rest of the epiblast. The proof that the streak is an organisation centre was also first given with the help of the *in vitro* technique;[1] it was shown that if two epiblasts were placed with their mesoderm faces together, each primitive streak could induce the formation of a neural plate, and probably an entire embryonic axis, in the part of the second epiblast against which it lay. Induction can also be performed by small pieces of streak grafted between the epiblast and endoderm of a host embryo.

Suggestions that part of the streak (the most anterior part, or Hensen's node) was an organisation centre also came from another quarter, namely from authors who claimed that differentiation could only be obtained from such isolated fragments of blastoderm as include Hensen's node. Wetzel[2] was the first to use this supposed fact as an argument to prove that Hensen's node is an organisation centre, but he later showed that in point of fact parts of the blastoderm not including Hensen's node are capable of further development, and he therefore withdrew his assertion.[3] It has, however, been repeated by most of the chorio-allantoic workers other than Hoadley, though Waddington, and more recently Wetzel, Waterman and Dalton,[4] using the chorio-allantoic technique, confirmed the fact that Hensen's node is not essential for development. It is to be noticed also, that even in the works of those who believe in the indispensability of the node, the size of the "node" (including the so-called "node field") grows larger as time goes on, and it becomes clear that less and less of the anterior part of the streak can be regarded as essential.[5] In any case, even if the node were essential for development, that would not prove it to be an organiser in Spemann's sense, which is defined by its capacity for altering the course of development of tissue on which it acts.

[1] Waddington, 1930, 1932.   [2] Wetzel, 1924.   [3] Wetzel, 1929 *b*.
[4] Dalton, 1935; Waterman, 1936; Wetzel, 1936.
[5] Rawles, 1936; Rudnick, 1938 *a*.