DEVELOPMENT IN ANIMALS

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THE most striking characteristic of living things is their ability to reproduce their kind. Among the lowest forms of animals, this process is relatively simple, consisting of the division into two of the original parent. This method involves little or no development, since each half has only to grow to adult size. Such animals, which belong to the group Protozoa, consist essentially of a mass of living material, the protoplasm, which is differentiated into two kinds, the more general outer substance called the *cytoplasm,* and a highly specialized inner area, the *nucleus.* The combination of a nucleus and its surrounding cytoplasm constitutes a unit of structure and function, which is called a cell by most biologists. Protozoa are examples of animals which consist of only one cell, or a few identical cells loosely bound together. This apparent simplicity of structure distinguishes them from all other animals which are built up of many such units, organized into tissues which are able to carry out particular functions—muscles, nerves, bones and so on.

Many physiological functions, the contraction of an isolated muscle, for example, can be explained in somewhat mechanical terms, since their basis lies in reactions which are amenable to the methods of modern physics and chemistry. Analysis of individual functional activities fails, however, to provide an adequate answer to the two major problems of biology:

(1) The problem of the manner in which an animal develops from a fertilized egg to its specific adult form.

(2) The problem of the process which coordinates the individual structures and functions of an animal, to produce a harmonious whole.

The history of the ideas and experiments which have attempted to explain the development of a chick from an egg, forms a long chapter, starting at least as far back as Aristotle and continuing to-day, still incomplete. The method of attack on this problem passed through the phases of philosophical speculation, observation and experiment, until now one may say that there is a serious attempt to interpret the wealth of experimental data on a unified theoretical foundation, which is free from the mechanistic interpretation of physical and chemical methods. Experimental work on the problem of development constitutes a branch of biology known as experimental embryology—a young science not yet 50 years old. The fundamental problem of experimental embryology may be outlined in order to make clear the generalizations which are to follow.

Every animal starts its life as a single cell, which consists of a mass of cytoplasm and a nucleus. This constitutes the egg cell, which in order to start development must be fertilized: i.e., the nucleus of the egg cell must unite with the nucleus of the sperm. As soon as the union of the male and female nuclei has occurred, the single cell divides into two cells; these in turn divide, and the process is repeated. This rapid cell division is the method of growth of the developing embryo, which consists at an early stage of a mass of similar cells, that gradually undergo changes which are visible to the eye, as the rudiments of various organs, such as the liver, heart and so on, appear and gradually develop into the fully formed animal. What is the stimulus which determines the precise course of development that results in an egg giving rise to a frog, a chick or a sea-urchin as the case may be? A brief description of some of the facts of animal development may help to clarify the problem.

All eggs have one characteristic in common: they are single cells. The differences in shape and size of various animal eggs depend on the presence or absence of protective membranes such as an egg shell, as well as on the quantity of reserve food present, which is called the *yolk*. The nucleus of the cell contains microscopic structures called chromosomes, which are known to transmit the parental characters to the offspring. The process of fertilization ensures the inheritance of the characters from both the father and the mother by the offspring, and the modern theory of heredity offers a satisfactory explanation of the resemblances and differences between parents and offspring.

The changes which occur at the time of fertilization are both structural and physiological, resulting in an initiation of activity in the cell, leading to division and rapid growth. First-formed cells are all alike, and the young embryo resembles a ball, the rubber of the ball being represented by a single layer of cells, the hollow of the ball being filled with liquid. This embryonic stage is called a *blastula*, and may consist of about 1000 cells, depending on the species of animal. In order that growth and development may continue, this hollow ball of cells becomes pushed in on one side, so that the hollow filled with liquid is almost obliterated, and a new cavity is formed where the pushing-in process (or invagination) took place. This stage represents an important milestone in the life of an animal, for it is at this period that the foundations of all the future adult structures of the organism are laid down. The stage is known as a *gastrula*, and it is already differentiated into the three layers of tissue which will provide the future animal with skin, brain, muscles, bones and blood, depending of course on the specific character of the organism concerned. The gastrula proceeds to develop rapidly, and one is able to see gradual foldings of the tissues to form rudimentary organs, such as the beginning of the nerve cord in a frog-embryo—this stage being referred to sometimes as a *neurula*. It is not necessary to go further with the details of development, for the progress in the development of the embryo consists in greater complexity of differentiation and specialization of the cells until the adult is formed.

One cannot fail to be impressed with the beauty of the process, as well as of the material, when one is watching it happen in the laboratory, especially if one is dealing with the transparent eggs of a common marine animal such as a starfish or sea-urchin, or even the more opaque egg of the frog. The most striking impression which one gains from the observation of living developing eggs is the rhythm in the changes which occur, and particularly the apparent aim with which the organism gains the goal of its adult form. This fact has been made use of by several writers, who see in the process of development a sort of "biological memory" which causes the organism to follow its precise and well-ordered scheme. Such an interpretation of this process is interesting, but, being entirely without proof, belongs to the realm of philosophy rather than science, and we may now turn to some experiments which give us some insight into this problem.

Suppose we take a sea urchin egg, fertilize it and allow it to develop until two cells are formed. These can be separated by gentle shaking, or by the addition of certain chemicals to the sea water surrounding the eggs. The two cells will fall apart, and if each is put into a separate dish of sea water, one is able to rear two perfect individuals of somewhat smaller size than normal; thus we get twins from one fertilized egg. Now we can go one step further, and allow another egg to develop until it consists of four cells. The same process of shaking is repeated, and the four cells separate and each will grow into perfect identical individuals; so we obtain quadruplets from one egg. The Dionne quintuplets represent a charming illustration of the principle under discussion. The conclusions which may be drawn from these experiments are:

(1) During the earliest stages of development of the sea-urchin egg, all the necessary materials which are required for the production of a new individual are equally distributed to the four

cells which have arisen by the two divisions of the original fertilized egg, since each quarter gives rise to a perfect miniature individual. Obviously the fertilized egg was not highly differentiated, or the separation of its parts would have resulted in abnormal development. So generalized is its organization that the same experiment may be extended to the eight cell stage, and occasionally perfect embryos develop from one of these separated cells. After that stage, however, separation of the cells results in various types of abnormal development.

(2) This type of experiment serves to answer the old controversy of the Preformationists, who believed that the egg contained a fully-formed minute replica of the individual, and that the process of development consisted merely in the growth or unfolding of the parts. Obviously if this were true, one could not separate the cells and still obtain a perfect individual.

The eggs of a sea-urchin and of many other invertebrate animals present a generalized structure, which acquires complexity during the course of development; and although these experiments give us some information regarding the initial condition of the egg and its behaviour during early development, we are still far from understanding how the various tissues and organs are functionally integrated to produce what we know as a living animal.

The work which has been described was carried out about 40 years ago by a German biologist, Hans Driesch, who was attempting to solve this problem of development. He came to the disappointing conclusion, that although he could analyse many of the individual steps in development, there was no mechanism which could explain the harmonious working of the whole organism, and he was led to postulate an external agency or "entelechy", which was incapable of further elucidation. Driesch considers the problem an insoluble one, and he has become the foremost upholder of the School of Vitalism among biologists. He has pursued his theory to its logical conclusion in his desertion of experimental embryology to become a professor of philosophy.

It might have been assumed from the results of the sea-urchin experiments that all animal eggs would yield similar results; but at the time that Driesch was carrying out his work, another German biologist, Wilhelm Roux, was laying the foundation of what is known as the "Mechanics of Development", which is the basis of all the modern work in experimental embryology.

Roux performed his experiments on the eggs of the frog, and obtained results quite different from those of Driesch, with regard to the earliest stages. Roux allowed the fertilized egg to develop until there were two cells formed; then he destroyed one of the cells by puncturing it, leaving it attached to the uninjured cell. The uninjured cell continued to develop as if its fellow cell were still alive and active—that is, the embryo which resulted from the uninjured cell was a half-embryo, lacking the other half which would normally have been supplied by the second cell. Other workers repeated Roux's experiments, but obtained occasionally a perfect embryo from the uninjured cell. These results were not as clear-cut as those of Driesch, but further experiments on the eggs of worms and snails, carried out by other embryologists chiefly in the United States, showed that there were two types of eggs. In those of the sea-urchin type, there is a generalized condition of organization which persists for a short time, each part of the embryo being capable of producing a perfect individual when separated from its neighbours. These separated portions would in the normal course of development behave as if they were parts of the whole; but if occasion demands it, they can take on the function of the whole, and this quality is expressed as a "power of regulation". In the type represented by the frog, the egg seems to possess a fair degree of initial differentiation, so that each part seems destined to form a particular region of the future adult; and if it becomes separated from its neighbours, it fails to accomplish complete development owing to parts lacking the regulative capacity of the more generalized type. The reasons for Roux's variable results have now been explained on the basis of the relation between the position of the division of the egg into two and the distribution of the egg contents. In other words, although a frog's egg looks round and perfect and uniform, it is really to be compared with a whole animal made up of many parts, like a lobster for example. There is only one plane in which a knife could divide a lobster into two symmetrical halves, namely a line running from the tip of the head to the end of the tail. If the line deviates, the knife will miss certain of the internal structures, and the two parts will not be perfect mirror images of each other. So it is with the frog's egg; if the line of division does not separate the egg's contents into two equal halves, the two cells will not be capable of individual development into a perfect embryo. The chances of this division into two symmetrical halves being accomplished are not very great either in the lobster or in the frog's egg, and this accounts for the different types of development which may occur in the development of the separated cells. The difference between the sea-urchin and frog types of egg is really one of the time at which differentiation occurs, rather than of anything of a fundamental nature, for the sea-urchin in the 8-cell stage behaves somewhat like the frog's egg in the 2-cell condition.

Roux's interpretation of development was directly opposed to that of Driesch. He was the leader of the Mechanistic School of thought, believing that materialism formed the only sound basis for exact science. Roux was in accord with the physiologists of his age, who found that many activities of the animal body could be explained in terms of chemistry and physics. Since Roux used the methods of physics and chemistry, it followed that his interpretation of development was expressed in the terms of those sciences, and consequently he was unable to answer the question in biological terms, although he used living material for his experiments. Roux's chief contribution to biology was his effort to direct attention to the part played by functional activity during development, which led to a great interest on the part of many workers in causal morphology. Roux tried to explain the integration of the activities of a developing organism without using the vitalistic idea of Driesch, but he was fully aware of the danger of regarding a living animal as a machine, and consequently offered no very conclusive interpretation.

The interpretation of development has always fluctuated between the two extremes of metaphysics and mechanism. To-day vitalism is no longer the creed of a working biologist, but it is probably equally true to assert that very few biologists believe that the total activity of an organism during either embryonic or adult life can be satisfactorily explained by knowing how each part behaves.

The work of Roux and Driesch resulted in the production of an unwieldly mass of information concerning the behaviour of developing organisms, both in normal and in abnormal environments, but it is only recently that a new light has been shed on a vexed question. This is represented by the work of Dr. Hans Spemann of Freiburg, the winner of the Nobel Prize for Medicine in 1935. Dr. Spemann, aided by a number of co-workers and students, has made use of a new technique which has clarified a number of points in development. His work is noteworthy not only for the results which he has obtained, but more for the reason that he has attacked a biological problem by using a biological method, and consequently he has obtained an answer which is biological in nature. Almost all the previous work in experimental biology assumes that an egg can be treated as a physico-chemical system. Consequently the activities were measured in these terms, and

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[1] Individual references are not given, but original papers from which this summary is taken appeared in *Die Naturwissenschaften*, Wilhelm Roux, *Archiv fur Entwicklungsmechanik der Organismen*, and other scientific periodicals published from 1924 to 1933.

the answer was expressed as if one had carried out an experiment on non-living material. This has resulted in a mass of information regarding isolated activities and parts of the animal, without any information as to the living organism as a whole. Spemann uses an animal for his experiments, asks a question of a purely biological nature, which is answered by the result of the reaction on the part of the living animal : thus he gets his final results in terms of biology, without the use of either physics or chemistry.

Spemann's enquiry concerns the question of differentiation during development. What is it that determines that certain areas will form certain structures rather than any other? His technique is that of grafting one part of an animal on to another and seeing the result. Grafting can be successfully carried out both on adult and on embryo forms of the newt, an amphibian and a close relation of the frog.

Grafting may be performed on members of the same species as well as between those of different species, a fact which is of great practical value, for it is then possible to choose two species of newts, which differ markedly in colour, making it comparatively easy to follow the fate of the grafted tissue which will differ in colour from the host animal in which it has been implanted. For example, one can graft the leg of a dark-coloured species of newt in the side of a light-coloured newt and *vice versa.* Both the grafts will grow normally, but they will be distinguishable by their colour. This can also be done with embryos of the newt (Triton), which was the material which Spemann used in his recent work.

Spemann's early work was concerned with much the same problem as that of Roux. He wanted to find out what the two first cells of a newt embryo were like. He tied a thread round the area of union of the two cells and drew the thread gently, constricting the two cells, without injuring them. He allowed them to develop, and found that he obtained a double-headed monster—an embryo with two well-formed heads joined to a single tail. This is similar to a case of incomplete twinning. If the thread is now pulled more tightly, the two cells can be separated, and two perfect, though small, embryos are obtained. This is similar to the separation of the two cells of the sea-urchin by Driesch. These cases emphasize the fact that under normal conditions the two cells behave as parts of the whole, but under exceptional conditions the separated cells can assume a greater capacity, and will behave as an individual giving rise to a perfect but small embryo. This fact led Spemann to consider the question of the capacity for development of different areas of a somewhat older embryo—whether all parts were the same, or capable of exerting a regulative capacity.

He used newt embryos in the gastrula stage (the pushed-in rubber ball stage) as well as the later stage, when it is known as a "neurula"—that is, the tissue which will give rise to the nervous tissue of the future adult has already been formed—which is referred to by Spemann as a "presumptive organ-forming region". The embryology of the newt is now so well-known that even the fertilized egg can be marked off into different "presumptive organ-forming areas", resembling a miniature map marked off into counties, each with its characteristic shape and size.

A brief description of the grafts which were performed and the results obtained will possibly give some idea of the scope of Spemann's work. It is impossible to try to convey the delicacy and beauty of the technical methods without illustration, so that must be assumed. Spemann used one newt with a coloured skin (*Triton taeniatus*) and the other with a white skin (*Triton cristatus*). The embryos of the two species also differed in colour, making it easier to see the transplanted portions. A piece of presumptive "brain" is removed from one animal and a piece of presumptive "skin" from the other and the two are interchanged, so that the presumptive skin of one is imbedded in the presumptive brain of the other. The embryos are allowed to grow for two days, and it is then seen that the presumptive brain has become skin in one embryo, and the skin has become brain in the other. This is surprising, and seems to prove in this case that the surrounding cells constitute the all-important controlling factor, so that presumptive brain tissue follows its destiny only if allowed to remain in its normal surroundings. This is true for all the other areas of tissue, at this very early stage (gastrula), and Spemann concluded that (a) these tissues are relatively indifferent at the beginning of this stage, (b) that there is some factor acting in the cellular environment that determines the definite fate of transplanted tissues. In addition it is interesting to note that although each transplanted tissue developed according to its environment, it retained its specific character of difference in colour. This type of experiment serves to emphasize the plasticity of tissues during the early stage of development, as well as the influence of position of the part in its relation to the whole.

With a later stage of development Spemann obtained entirely different results. Such later stages of the embryo exhibit a high degree of differentiation in certain regions, which may be illustrated by the following experiment. An area of the embryo known as the "dorsal lip", which will normally give rise to such structures as backbone and muscles, is removed and placed in another embryo in the region of the future skin. When the embryo containing the graft was allowed to grow for several days, it was found that practically a complete second individual had grown from this small piece of dorsal lip and was now lying attached to the host embryo. This new individual attached to its host consists of the grafted material, but also partly of the cells of the host, that have been induced to adapt themselves to these cells. The dorsal lip tissue is known as an "organizer", because it has induced the host tissue to develop into a number of tissues from what would normally have been nothing but skin.

A region of tissue which has this peculiar power of induction is called a "centre of organization" and the analysis, which has been very completely carried out, shows in the later stages of development there are several of these "centres of organization", which vary in their ability to produce tissues—some being able to induce a whole new embryo, others only certain structures.

The results of this experimental work emphasize clearly the necessity of regarding a developing organism as a unit, rather than a mosaic of separate parts and functions, for it is only when all parts of the animal are in their normal relations to each other and to their environment that one sees a "living organism". A disturbance of any one factor brings about a change or even an abnormality. It is obvious not only that the attainment of the adult form is orderly and precise, but that the definite advances which occur at specific embryonic stages are essential for future development. The definite embryonic stages can be regarded as "organisms as a whole" just as much as the adult. The importance of this work lies as much in its theoretical implications as its experimental data, for it constitutes as admirable approach to the biological conception of "the organism as a whole" as a basis for the formulation of a theoretical biology which is independent of the methods of other branches of science.