CHAPTER VI

FORMATION OF THE GERM-LAYERS

THE period that we are now about to examine is marked by extensive movements of parts of the segmented egg as a result of which the organs are formed. During the segmentation-period the cells retain, as we have seen, the position in which they arise, but with the appearance of the blastopore a new period is initiated in which extensive movements of cells and groups of cells take place.

HIS'S EXPERIMENTS WITH ELASTIC PLATES

His ('94), from his studies of the behavior of elastic plates, has concluded that many of the phenomena of the developing embryo are the mechanical result of the tensions set up in the different layers. In the embryo the shoving, compression, or extension is supposed to result from the unequal growth of different parts. When a cell-plate lifts itself up into a fold, as a result of more rapid growth in that region than elsewhere, there is present on the concave side a positive tension ("Druckspannung") and on the convex side a negative tension. Under these conditions the cells become conical, *i.e.* they are small on the concave side and broad on the convex side of the fold. Each embryonic cell tends of itself to become spherical and only the surrounding conditions, resulting from the growth of surrounding parts, determine the shape of each cell at any period of development. His has tried to explain many of the changes taking place in the early embryo as the result of this simple folding principle. The inrolling of the medullary plate, the formation of the eye-outgrowth from this plate, the formation of the mouth-cavity and the gill-slit-folds, etc., are examples of some of these changes. His pointed out how closely the forms taken by many of these structures in the embryo resemble the folds that can be produced mechanically by pulling out or pushing in a thin elastic plate of rubber. If this interpretation is true, it means that at different periods in the development, regions of more rapid growth appear, now here, now there, and as a mechanical result of the conditions present, such structures as the medullary folds, the eye-outgrowths, etc., are produced. The cells change their shape in response to surrounding conditions, *i.e.* they do not by their individual activity or movement change their shape to produce the successive changes of the embryo, but the shape of many cells is changed as the result of growth or increase in mass of certain regions. For instance, a cell becomes conical not through its own initiative, but because the surrounding pressure forces it into a conical shape.

THE FORMATION OF THE EMBRYO BY CONCRESCENCE

The period of overgrowth of the blastopore when the so called process of gastrulation is going on has been described in Chapter V. We may now follow the changes that take place in the interior of the egg during that time.

When the dorsal lip of the blastopore appears, the cells have shown little tendency to arrange themselves into sheets or layers. However, even when the segmentation-cavity is covered by a roof of small cells, the cells of the outer layer have begun to flatten against one another and to form a thin layer of cells over the outer surface of the black hemisphere. In the lower hemisphere the larger white cells do not show such an arrangement. In the equatorial region, where the black and white cells meet, a careful examination of sections will show that there exists a more or less defined ring of cells stretching around the embryo, forming a broad zone (Fig. 15, D). The *inner* cells of this ring contain a good deal of pigment around the nuclei. The yolk-granules of these inner cells are smaller than the yolk-granules in the large white cells of the lower hemisphere, and the cells of the ring seem to contain also a larger amount of clear protoplasm. This inner zone of cells passes, on the one hand, by insensible gradations into the cells of the outer surface of the ring and internally it is continuous with the inner region of large yolk-cells. *This ring of cells, as subsequent development shows, is the beginning of the embryo, and the ring itself is composed of the material which subsequently forms the central nervous system, the mesoderm, the notochord, and a part of the endoderm.* An understanding of the subsequent development depends on a knowledge of the changes that take place in this ring.

The material of the ring is intimately involved in the movements that take place during the overgrowth of the lower hemisphere by the lips of the blastopore. During this period, we must picture to ourselves the ring as rising up and drawing together over the lower white hemisphere, so that ultimately it leaves its equatorial position and its halves come together to form the embryo. (Fig. 24, A, B, C.)

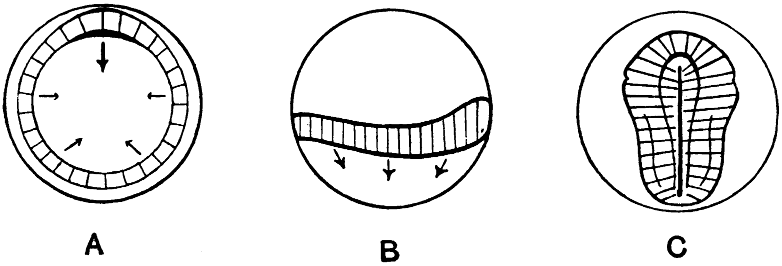

FIG. 24.—Diagrams illustrating germ-ring and concrescence of lips of blastopore.

As the dorsal lip of the blastopore progresses over the white hemisphere, its progress is due to the movement and fusion along a meridian of the material of the equatorial ring. We are to think of the material of the ring as moving toward the middle line from the *right* and *left* sides (for with the establishment of the dorsal lip the *ring* becomes bilateral) and fusing continuously in the dorsal lip (Fig. 24). The advance of the blastopore is merely the expression of the absorption into its dorsal lip of the material of the two sides of the ring. As soon as the material from the sides reaches the median line in the dorsal lip of the blastopore, it remains stationary and new material is added behind that just laid down. The material of the equatorial ring is thus carried into a meridian of the egg. With the disappearance of the yolk-plug below the surface, the final stages of overgrowth are completed. The ventral lip of the blastopore has moved somewhat forward, as previously explained, and this slight forward movement probably takes place by the growth toward the median line of the material at the sides of the ventral lip.

There are other changes closely bound up with the preceding phenomena and, although these changes take place simultaneously, it will be necessary first to consider them separately, and then to try to combine them into a single statement. The changes involve, 1) the formation of the archenteron, 2) the progression of the blastoporic rim over the lower hemisphere, 3) the origin of the middle layer or mesoderm.

THE FORMATION OF THE ARCHENTERON

1) When the dorsal lip appears, certain cells pull away from the surface, leaving their outer pigmented ends exposed for a time (Fig. 15, D, Fig. 12, H). These cells are near the border-line between the black and white regions, but lie distinctly amongst the white cells. The next change involves the sinking in beneath the surface of the region in which these cells are present. The dorsal lip of the blastopore now begins its movement over the lower hemisphere. From the surface we can see that the crescent becomes longer and longer, the horns extending outwards along the black-white border but well within the white. The same changes that took place where the dorsal lip first appeared, now take place also wherever the crescent extends. First certain superficial cells pull into the interior of the egg leaving only their pigmented ends at the surface, and then this area of pigment sinks below the general surface. Simultaneously the edges of the blastopore roll over the inturned (invaginated) cells. The same changes also take place at the posterior or ventral lip of the blastopore, when the two horns of the lateral lips have met there. It is necessary to examine sections that have been cut in several planes in order to follow the changes that take place during the further overgrowth of the blastopore. If we examine a median longitudinal (sagittal) section at the time when the dorsal lip has just begun to roll over, we find (Fig. 25, A) that a narrow space is left between the dorsal lip and the surface of the lower hemisphere over

which the dorsal lip has begun to roll. We find, at the upper end of this crevice, the pigmented ends of those cells that were previously at the surface. During later stages the space, which we may at once speak of as the archenteron, becomes longer, due to a further progression of the dorsal lip over the white hemisphere. If the section were taken somewhat to one side of the median line, the length of the archenteron would be found to be less than in the median line, because the rolling in has been relatively less. If we make a section at right angles to the last in the plane Y–Z, in Fig. 19, A, we cut the two horns or ends of the crescent. The cavity on each side is just beginning, owing to the smaller amount of closing in from the sides of the lateral lips of the blastopore. (Fig. 19, B.)

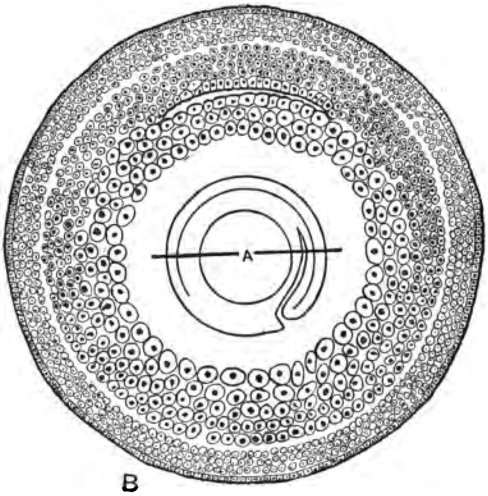

FIG. 25.—A (small figure inside B). Longitudinal section through young embryo. B. Cross-section of last. (After Schultze.)

A section at right angles to the last section in the plane of the line in Fig. 25, A, is shown in Fig. 25, B. The archenteron is seen in the upper part of the section. Its upper or dorsal wall is made up of small cells, while its floor is formed of large cells filled with yolk. The segmentation-cavity fills the centre of the section.

During the time when the yolk-plug is withdrawing from the surface, the segmentation-cavity becomes smaller, owing, without doubt, to the intrusion of the large yolk-mass into its interior, and finally, when the archenteron begins to open, the segmentation-cavity is almost entirely obliterated. The segmentation-cavity is thus utilized by the embryo, for into this cavity is pushed the yolk-mass as the latter is overgrown by the blastopore-lips. This statement does not necessarily imply, however, that the segmentation-cavity was prepared especially in view of the subsequent changes.

It will be seen from the foregoing account that the walls of the archenteron are formed as the blastopore closes in. The floor of the archenteron (Fig. 25, B) is nothing more than the surface of the lower white hemisphere that is overgrown. The origin of the roof and sides of the archenteron is somewhat difficult to understand. We have seen that around the crescent of the blastopore certain cells have pulled in, leaving a depression on the surface. It is impossible to say just how far the cells that pull in continue to be drawn inward, because simultaneously the lips of the blastopore roll over. This brings us to a discussion of the second topic.

THE OVERGROWTH OF THE BLASTOPORIC RIM

2) There are at least two ways in which we may think of the closing in of the lips of the blastopore, *i.e.* there are two ways, either of which might explain the covering of the white by the black cells. We may think of the *free edge* of the blastopore as growing toward a middle point. Or we may imagine that the lateral and dorsal edges actually roll in toward the middle line. The latter process seems to be that which probably takes place, for Jordan ('93) has seen the outer dark cells actually rolling over and into the archenteron in the living egg.

The dorsal and lateral walls of the archenteron will then be formed in part, or entirely, from those cells of the surface that have rolled in and have come to lie beneath the surface. These are the cells, therefore, that have been at one time situated at the surface of the embryonic ring, and inasmuch as the advance of the dorsal lip takes place very largely by the fusion of the lateral lips, it follows that the material for the greater part of the dorsal wall of the archenteron comes from cells at one time on the outer surface of the egg. I am inclined to think that at first there is also an actual in-pulling of cells along the blastoporic rim so that cells at one time below the outer surface come also to stand, later, at the sides of the archenteron, *i.e.* where the dorsal and ventral walls meet.

THE ORIGIN OF THE MESODERM

3) It is difficult to give an account of the method of development of the mesoderm, because there are almost as many different descriptions of the process as authors who have described it. I have without hesitation set aside those accounts where the author has transparently sought to find his preconceived theories demonstrated in his drawings of the sections of the embryo. In the second place, several of the more recent accounts have started out, I think, with a false conception of the position of the embryo on the egg and its method of formation, hence in these accounts the *method* of the formation of the mesoderm is likely to be erroneously described, although in several cases the actual drawings of the sections have been, I believe, accurately made. I have followed as far as possible those interpretations that are in conformity with the experimental results relating to the growth of the embryo. Certain abnormal embryos, to be described later (Chapter VII), that first appear as a ring around the egg throw, I think, also much light on the subject.

The cells that are to form the mesodermal layer are present at the time when the dorsal lip of the blastopore has first appeared, and even just prior to that time. The innermost of those cells forming the ring around the egg are the cells that become the mesoderm (Fig. 19, B). These cells are carried up to the median dorsal line of the embryo by the closure of the blastopore (Fig. 24, A, B, C). They will then be found forming a layer or sheet of cells (Fig. 25, B) that separates itself on the outer side from the thick layer of small ectodermal cells (that has been simultaneously lifted up) and that is separated on the inner surface, but not very sharply if at all, from the dorsal and dorso-lateral walls of the archenteron. A continuous sheet of tissue is formed in this way over the dorsal surface stretching across the middle line. According to some accounts, the fusion of this mesoblastic sheet with the endoderm is much closer in the mid-dorsal line than on each side. We may, however, think of the mesodermal layer and endodermal layer as coming up *together* to the median line from the sides, so that we are to think of the mesodermal and endodermal cells as being together from the beginning.

DIFFERENT ACCOUNTS OF THE ORIGIN OF THE ARCHENTERON AND MESODERM

Before following further the fate of these concentric coats or layers of cells, the so-called "germ-layers," we may for a moment examine some other descriptions that have been given as to the method of formation of the archenteron in the frog. The most common view of the method of gastrulation of the frog has been that a process of *invagination* takes place at the dorsal lip of the blastopore. This process is supposed to be brought about by the drawing inwards and upwards of a fold of the outer wall, so that a blind sac forms. As this presses forward into the yolk, the latter pushes before it and fills up the segmentation-cavity. At the same time the mesoderm is described as growing forward from the region of the blastopore over the dorsal surface of the embryo.

Other authors represent, however, the dorso-lateral edges of the archenteron proliferating cells along the two sides to form the mesoderm, while in the mid-dorsal line a solid block of *endoderm* cuts off to form the notochord. Hertwig has gone so far as to affirm that at the dorso-lateral edges of the archenteron there are traces of a pair of lateral pouches along each side, and that these give rise to the cells that push in between the ectoderm and endoderm to form the middle layer.

Robinson and Assheton ('91) assert that the old account of the formation of the archenteron by invagination is entirely erroneous, and that the cavity of the archenteron owes its existence to a process of progressive splitting or separation of the large yolk-cells of the lower hemisphere, and that this splitting extends up into the yolk beneath the upper hemisphere. The dorsal lip of the blastopore remains approximately stationary where it first formed, and the anus develops around this point.[1]

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[1] In a later account Assheton ('94) has much altered his former view. He describes only the anterior end of the archenteron as formed as a split amongst the endoderm-cells, while the posterior third of the archenteron is, he thinks, the result of the overgrowth of the dorsal and lateral lips of the blastopore.

Both assumptions are, I think, erroneous, as a study of the changes that take place in the dorsal lip will convince any one who will take the trouble to follow in the living egg the method by which the closure of the blastopore takes place.

LATER DEVELOPMENT OF THE MESODERM AND ORIGIN OF THE NOTOCHORD

Schultze ('88), who has studied the formation of the middle germ-layer of the frog, has given an accurate account of the condition of the mesoblast in the embryo during the period of overgrowth of the blastopore. He has done this, too, despite the fact that he believes the embryo of the frog to be formed over the upper or black hemisphere of the egg. This belief has not, however, in my opinion, vitiated in any degree his description of the position of the mesoblast after its formation. I have, therefore, reproduced his figures in Fig. 26, A–E.

If a cross-section be made through an embryo (in the plane of the dark line of Fig. 25, A) at the time when the blastopore has assumed a crescentic shape, we find over the surface of the section a thick envelope of ectoderm. The ectoderm is at this time composed of about four layers of cells (Fig. 25, B). In the outermost layer the cells are columnar in shape. In the centre of the section there is a large segmentation-cavity surrounded by large yolk-bearing cells. The archenteron, as seen in cross-section, is a large, arched cavity, its lower wall formed by yolk-cells and its dorsal wall covered by a layer of small cells showing a tendency to become flattened against one another. Above the upper wall of the archenteron, and between it and the ectoderm, is a thick layer of cells. This layer stretches out on each side of the embryo as a lateral sheet, but the edges of the sheet merge insensibly into the yolk-bearing cells at the sides. Where this middle layer (mesoderm) is sharply defined, we can easily distinguish its cells from those of the endoderm, for the mesodermal cells are smaller and pigmented. At the free edge of the sheet it becomes, however, impossible to distinguish between the cells of the mesoderm and of the endoderm.

If we examine a complete series of sections through this embryo, we find that the layer of mesoderm is inserted between ectoderm and yolk-cells over all the posterior half of the embryo. There is a small antero-ventral region into which the mesoderm does not extend. At a point posterior to the section described above, we find the mesoderm extending much farther *ventrally*, so as to nearly encircle this region of the embryo. The blastopore is completely encircled by the sheet of mesoderm.

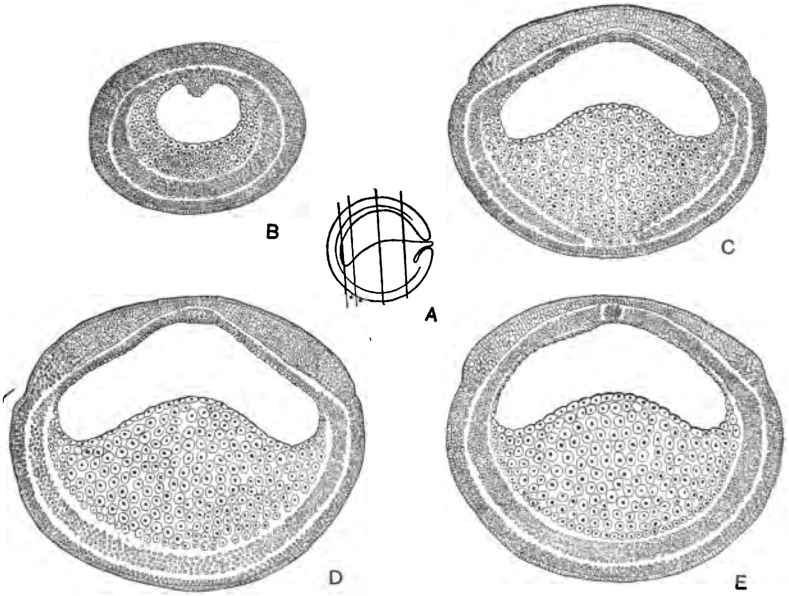

FIG. 26. — A. Longitudinal section through a young embryo of Rana. B, C, D, E. Cross-sections of last in planes of lines in A.

Cross-sections through an older embryo are drawn in Fig. 26, B, C, D, E. The embryo has flattened along the mid-dorsal line. The ectoderm has become thinner along this line, where a faint groove can be seen on the surface of the living egg, — the primitive groove. On each side of the mid-dorsal line, the ectoderm is somewhat thicker than before, and the cells are more closely packed together. The ectoderm over the surface of the embryo consists of an outer layer and of several inner layers of cells. The cavity of the archenteron has opened out and is very large.

As before, its ventral wall is composed of larger and yolk-bearing cells. Above and laterally the walls are formed of smaller cells. The latter have now arranged themselves in a definite layer, and have become somewhat flattened (Fig. 26, B, C, D). This layer is also sharply separated from the mesoderm. The mesoderm, as compared with its previous condition, has undergone important changes. It has extended further ventrally, and has met from the right and left sides in the mid-ventral line along most of the ventral surface. Over the dorsal and dorsolateral walls of the archenteron it forms a thinner layer of cells than in the earlier embryo (Fig. 25, B).

There is still a ventral region of the embryo where the ectoderm and the yolk-cells are in contact, *i.e.* a region into which the mesoderm has not extended (Fig. 26, C). The medullary plate is seen in cross-section. It will be noticed that the plate is much thinner in the mid-dorsal line than at the sides. On each side the medullary plates show a differentiation into two parts. The most lateral and ventral edge of the plate is formed of cells less closely held together than those nearer the mid-dorsal line. This mass of rounded cells is the beginning of the neural crest.

The mesoderm in the mid-dorsal line is thickened in the posterior sections. According to some writers, this median mesoderm has *always up to this time remained closely fused with the layer of endoderm beneath it.* It marks the beginning of the notochord.

The formation of the notochord takes place from behind forwards, so that in the same embryo different stages of its development may be found (Fig. 26, D, E).

The account given above of the formation of the notochord is not generally accepted, particularly since the formation of the notochord from the endoderm is the method followed by many, perhaps by all other vertebrates. That a median mass of tissue stretches at first across the dorsal median wall of the archenteron in the frog cannot be denied, but many embryologists have preferred an interpretation different from that which I have followed. It is affirmed that there is always a *closer connection between the endoderm* and the tissue lying above it in the dorsal median line than between the endoderm on each side of the mid-dorsal line and the mesoderm. Further, it is said, that the cord of cells in the median dorsal line remains for a longer time connected with the mid-dorsal endoderm than does the mesoderm at each side with the lateral endoderm, and that the notochord separates from its lateral connections (right and left) with the mesoderm, while it still remains for a time closely fused in the mid-line with the endoderm.

In the newt and in other urodeles the endoderm in the mid-dorsal line thickens and bends upward to form a longitudinal fold. The fold pinches off from the endoderm and forms a cord of cells, — the notochord. In the posterior end of the toad's notochord the same method of development may be seen sometimes to take place.[1]

With such clear evidence of the method of formation of the notochord from endoderm in the newt, it is not surprising that embryologists have attempted to interpret the changes that take place in the frog in the same way. The main difficulty arises from an unwillingness on their part to derive the notochord from the so-called middle germ-layer, or mesoderm. The question therefore turns, *for them*, on what they will call the middle layer in the frog, and what not the middle layer.

Since, however, all the cells in this region have had a common origin, the question is perhaps a trivial one; for we cannot doubt, I think, that had some of the cells in the middle line passed a little to one side or the other of the median line, they would have been capable of becoming mesoderm, and, *vice versâ*, had some of the lateral cells come to lie nearer to the middle line, then they would have taken part in the formation of the notochord.

The notochord separates entirely from the mesoderm and endoderm, and becomes rounded in cross-section. On each side of the notochord the mesoderm becomes thicker, as is shown in Fig. 42. The final stage in the closure of the medullary folds and the changes that take place in the mesoderm will be described in a later chapter.

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[1] Field ('95).