CHAPTER V

EARLY DEVELOPMENT OF THE EMBRYO

In the preceding chapter the cleavage of the egg has been described to the period when the blastopore is about to appear on the surface. During the subsequent development the cells continue to divide, so that at no time can the cleavage or the cell-division be said to cease. At each successive stage the number of cells is greater than in the preceding stage. This statement does not imply, however, that the formation of each new structure is introduced by new cell-divisions in the region where the change is about to begin, because many changes take place in regions where cell-division is not more rapid than elsewhere.

The spherical form of the "egg" or young embryo is soon lost. In the present chapter we shall follow the changes that can be seen taking place on the exterior of the living embryo; and in the following chapter we shall attempt to make out the movements of cells and groups of cells that take place in the interior of the embryo during this period.

The Blastopore

On that side of the egg where the smaller cells are found, a short horizontal line of pigment[1] appears amongst the white cells below the equator of the egg (Fig. 12, I). This line marks the beginning of the archenteron, and the cells bounding the upper or darker side of the pigment-line form the dorsal lip of the blastopore. The dorsal lip becomes crescentic in outline, with the concavity of the crescent turned toward the white hemisphere (Fig. 19, I, II). If the living egg be watched, it

[1] There is a great deal of variation at first in the shape of the blastopore.

will be found that changes take place at this time in the blastoporic region with great rapidity.

Pflüger has described ('83) these changes, and we may follow his admirable account, subsequently adding other facts that have since been discovered. The eggs which Pflüger studied[1] were taken from the uterus at twelve o'clock midday, and placed in a row on a thick glass mirror. The mirror was then put into a dish, and water added to the depth of 2 mm. In this way, owing to the reflection of the lower pole by the mirror, both hemispheres of the egg could be watched. During the night, when the temperature was low, the eggs developed more slowly. At six o'clock in the morning the thermometer stood at 16° C. At this time the eggs showed on the lower hemisphere, and in the upper fourth of that region and therefore just beneath the equator, the first trace of the dorsal lip of the blastopore. By ten A.M. the long horizontal split (dorsal lip of the blastopore) had become distinctly marked as an indentation of the surface of the egg. At eleven A.M., the dorsal lip had moved somewhat further below the equator of the egg, *i.e.* toward the lower pole. The "split" is now broader, and its corners turned down so that it forms a crescent, with the lower pole of the egg-axis as its middle point. The diameter of the crescent is to the egg-diameter as 2 : 3. From the corners of the crescent a furrow continues to extend on each side around the white hemisphere. The progress of the dorsal lip toward the lower pole is not due to a rotation of the egg *as a whole*, but to the *migration of the dorsal lip over the white hemisphere*. At half-past twelve o'clock (twenty-four hours after fertilization), the dorsal lip has progressed further toward the lower pole. The crescent has at the same time extended so as to form a half-circle whose diameter is somewhat less than in the preceding stage. It stands now in relation to the diameter of the egg as 1 : 2.

By one o'clock P.M., the semicircle forming the dorsal and lateral lips of the blastopore has extended so as to form a complete circle (Fig. 19, A, IV). The white yolk-cells protrude from the centre of this circle and form the so-called

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[1] Bufo cinereus.

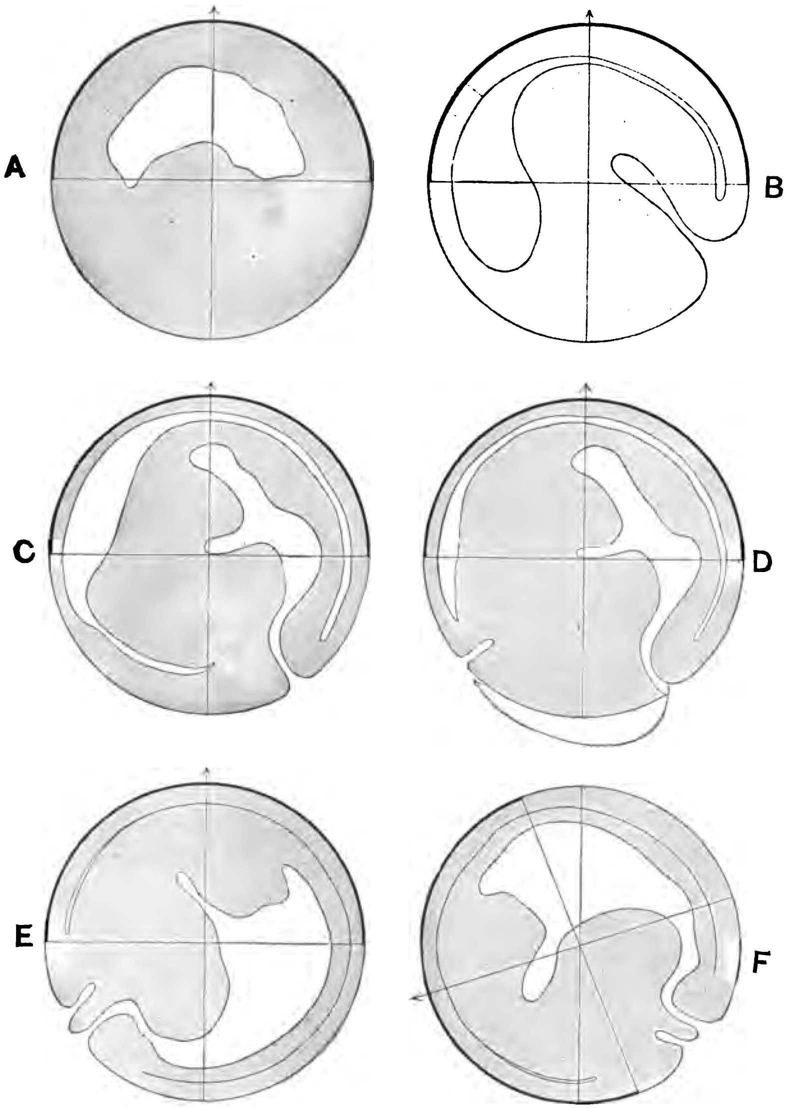

Fig. 18.—Diagrams to show extent of movement of dorsal lip of blastopore and rotation (F) of embryo. (After Pflüger.)

yolk-plug. The diameter of the circle around the yolk-plug is still smaller than before. At 2.15 P.M., the opening containing the yolk-plug — the so-called opening of Rusconi, or blastopore — is still smaller. The periphery of this circular blastopore is deeply pigmented. At 4.15, the opening is further reduced and measures no more than one-eighth of the diameter of the egg. The blastopore will now be found to have progressed so far that it again lies just beneath the equator of the egg, but on the side of the egg opposite to that at which the dorsal lip first appeared. We can summarize by saying that the dorsal lip of the blastopore has moved over a meridian of the egg from a point near the equator across nearly to the opposite point of the equator. The movement takes place over the lower white hemisphere, and during the process the position of the egg remains unchanged. The arc traversed by the dorsal lip of the blastopore is, however, not as much as 180 degrees, because it started below the equator and does not quite reach the equator at the opposite side. But the arc is certainly more than 90 degrees, and varies in different eggs.

So far we have traced the history of the blastopore from six o'clock in the morning to 4.15 in the afternoon of the same day. Then a remarkable process begins. The blastopore moves back as a whole in exactly the opposite direction until, at 7.45 in the evening, it has come back to the point from which it started in the morning. This reverse movement of the whole blastopore is brought about by quite a different process from the first movement of the dorsal lip. *The whole egg rotates around a horizontal axis.*[1]

The overgrowth of the lower pole by the dorsal and lateral lips of the blastopore has covered the lower hemisphere with cells that do not contain as much pigment as do the cells that lie around the upper pole, *i.e.* the original black hemisphere. Hence when the egg rotates as a whole in the way just recorded, a somewhat lighter area will be carried into the new upper hemisphere, while the original upper hemisphere will now come to lie nearly on the lower side of the egg. In the lighter upper region, as we shall soon see, the central nervous system develops. The rotation of the whole egg appears to take place through 180 degrees, although it is possible that the

[1] An axis at right angles to the median plane of the later embryo.

central nervous system may also grow forward somewhat so that the actual rotation is not great.

Perhaps the whole process may be made clearer by reference to a series of sections through the egg. These are taken through the meridian that corresponds to the middle plane of the body; it therefore passes through the upper and lower poles (Fig. 18). The arrows indicate the primary axis. The dorsal lip of the blastopore has formed in Fig. 18, B, and in Fig. 18, C, D, the migration of the lip has gone further over the lower hemisphere. The ventral lip of the blastopore has also formed. Figure 18, D, corresponds to a stage in which the blastoporic circle is completed. In Fig. 18, E, we see that the dorsal lip has travelled further over the lower pole toward the ventral lip. Finally,

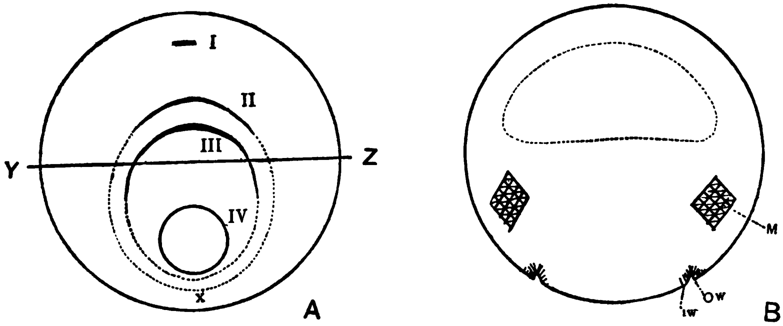

FIG. 19.—A. Diagram to illustrate overgrowth of dorsal lip of blastopore. I-IV represent different stages. B. Diagram of cross-section through Z-Y of A, to show lateral lips of blastopore, and mesoderm (M), and (IW) inner wall of archenteric pit. OW. Outer wall.

in Fig. 18, F, the egg is represented as having rotated as a whole to bring the embryonic portion above.

The changes that take place during the closure of the blastopore are perhaps more clearly shown by the following experiment. By means of a fine pointed needle it is easy to puncture the egg slightly at any given point. When the outer surface of the egg is pierced, there follows a protrusion of material as soon as the needle is withdrawn. At other times when the surface is only *indented* (*not* pierced) by the needle, there follows a blunt protrusion of material and the surface remains unbroken. In the latter case the marks do not always last as long as do those produced by the first method, but as less harm is done to the egg, one can often get more satisfactory results.[1]

If when the first trace of the blastopore appears on the surface of the egg (Fig. 19, A), a slight injury is made to the surface of the white hemisphere at the side opposite the blastoporic lip, *i.e.* at a point 150 degrees from the dorsal lip of the blastopore (Fig. 19, A, at *x*), we shall find that in the course of four hours the blastopore will form a crescent, and that the distance from the dorsal lip to the point of injury is much less than at first (Fig. 19, III). A circular line of pigment in the white hemisphere shows the line along which the lateral and posterior lips of the blastopore will appear. It will be seen in this case, that the point of injury lies therefore outside of the yolk-plug, *i.e.* posterior to the ventral blastoporic lip.

In the course of four hours more it will be found that the circular blastopore is much smaller than before, and that the dorsal lip now lies much nearer to the point of injury (Fig. 19, IV). The dorsal lip has travelled over more than two-thirds of the original distance from its starting-point to the point of injury. By making a new experiment with an egg that has reached this stage of development, it will be found that when the outlines of the blastopore have become sharply defined, the later closure takes place at nearly an equal rate from all points of the circumference, perhaps, however, still somewhat more rapidly from the dorsal lip backwards.

Where the diameter of the circle representing the outline of the egg equals 27 mm.,[2] the distance between the blastopore and the injury measures 24 mm. In the first four hours the blastopore moves through 8 mm. In the next four hours it travels through 7 mm., and is therefore now only 9 mm. from the point of injury. By this time the blastopore is circular in outline, and the injury lies just outside (2 mm.) of the circle. The blastopore now measures 7 mm. in diameter. Assuming that from this time forward the blastopore grows together at an equal rate toward its centre, then the *dorsal lip* will pass over about one-half of the diameter of the blastopore, or 4 mm.

[1] This method can be used only with great caution.

[2] The numbers refer to the measurement of the figure, and not to the egg itself.

The dorsal lip has passed then, in all, through 19 mm. of the white area; the ventral lip (from behind, forward) through 3 mm.

If the region overgrown by the dorsal lip be compared with the length of the medullary folds which soon appear in the same region of the embryo, it will be found that the latter, when they first appear, are somewhat longer than the region overgrown. If, however, we deduct from the length of the medullary folds the thickness of the anterior connective that joins the right and left sides of the nerve-plate, we shall find that the remaining length of the medullary folds corresponds very closely with the length of the region overgrown. We must, therefore, conclude that the anterior connective lies just in front of the point at which the first trace of the dorsal lip of the blastopore appeared.

We have assumed the point of injury to be a fixed point and the overgrowth to be due to the progress of the dorsal lip. It might equally well have been assumed that the overgrowth was only apparent and was produced by the sliding forward of the whole of the white area beneath the dorsal lip of the blastopore. The end-result would be the same, but the process different. There can be no question, however, that the movement is really due to the progress of the dorsal lip. Other experiments where two or more points of the surface are injured show very conclusively that the movement is a backward growth of the rim of the blastopore.

Comparing the statements made above with those of Pflüger, it will be found that they differ in three unimportant respects. The rapidity of the overgrowth of the very early stages, before the complete establishment of the crescent, was not noted by Pflüger. The distance travelled by the dorsal lip, as just described, is somewhat less than that given by Pflüger. Pflüger thought that the dorsal lip moved over about 180 degrees, but added that the amount of the movement differed in different individuals, and was probably between 90 and 180 degrees. My own results make the region of overgrowth about 120 degrees. From Pflüger's figures we are led to believe that the whole blastopore after the establishment of its ventral lip continues to move somewhat nearer to the equator of the side nearest to the ventral lip. If this really does take place, in the way shown by Pflüger's figures,[1] it can only be due to a slight rotation of the egg as a whole in this direction, for experiments show that the entire movement of the ventral lip is forward, *i.e.* toward the dorsal lip.

The yolk-plug is finally withdrawn into the interior of the egg and the blastopore remains as a round or often somewhat elongated opening. Its subsequent changes we shall follow later.

EXTERNAL CHANGES AFTER THE CLOSURE OF THE BLASTOPORE

Let us next examine the changes that appear at this time in the region that now lies anterior to the blastopore and on the upper surface of the egg. There is much variation in the early stages of development of the embryos of a given species, and in different species the variations are even greater. The differences in level of different regions are the result of movements of the ectoderm. To see these to best advantage, the *living* egg must be placed in the direct sunlight, and the surface studied with low powers of the microscope.

An embryo in which the yolk is still exposed is shown in Fig. 20, A. Passing forward from the yolk-plug over the upper surface of the egg is a broad groove, the so-called "primitive groove." At the anterior end of the primitive groove is a circular elevation. On each side of the primitive groove, at IM, the inner medullary folds are seen. Outside of these we find a depression, and farther on each side, at EM, the outer medullary folds. A sickle-shaped depression lies just in front of the blastopore.

A later stage of the same embryo is shown in Fig. 20, B. The primitive groove is narrower, the medullary folds are more distinct, and anteriorly a continuation of the lateral folds has formed. This will later be called the head-fold. Anteriorly and laterally, there is formed on each side a lateral extension of the medullary plate, the so-called "sense-plate."

The medullary plates now begin to roll in, producing a deep furrow, the medullary furrow with the primitive groove at its

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[1] Pflüger ('83), Pl. II., Figs. 4 and 5 (see Fig. 18, F).

bottom (Fig. 20, C). The lateral sense-plate is split into an anterior and posterior part on each side. The more anterior part may still be called the sense-plate, SP, and the posterior, the gill-plate, GP.

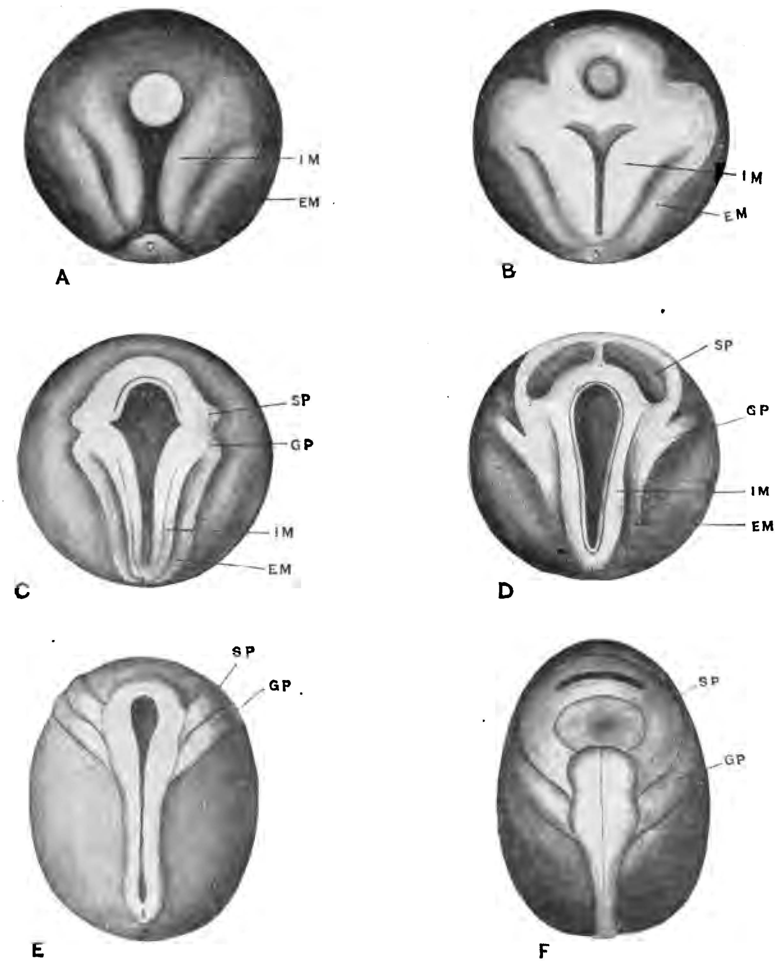

FIG. 20.—Surface views of young embryo of Rana. (After Schultze.) IM. Inner medullary plate. EM. Outer medullary plate. GP. Gill-plate. SP. Sense-plate. BP. Blastopore.

A later stage is shown in Fig. 20, D. Here the sense-plate is found to have extended laterally and forward, and the two sides have met in front of the medullary folds. The gill-plate is also seen, but the outer medullary folds are no longer conspicuous. The inner medullary folds are closing in to form a tube. The blastopore is reduced to an elongated slit-like opening.

A still later stage is drawn in Fig. 20, E. The outline of the whole egg is now elliptical, with the long axis in the direction of the long axis of the embryo. The medullary folds are also much longer, and have approached each other in the middle line. A deep furrow lies between the two halves. The

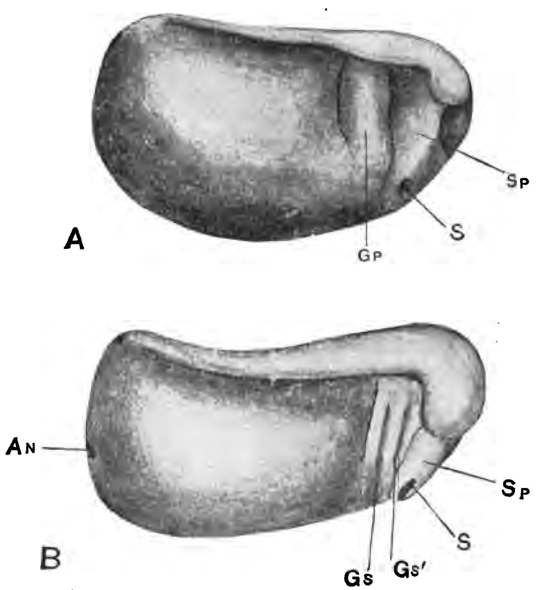

Fig. 21.—Embryos of Rana. (After Schultze.) Gs, Gs′. Two gill-slits. Gp. Gill-plate. Sp. Sense-plate. S. Suckers. An. Anus.

folds have more nearly approached at the middle of their length, and are more widely separated at the anterior and posterior ends. At the posterior end the medullary folds are overarching the small elongated blastopore. The sense-plates and the gill-plates are distinctly visible.

The medullary folds now fuse along their whole length, leaving, as we shall see, a central canal, which is the overarched medullary furrow. The elongation of the embryo continues as seen in Fig. 21, A. The anterior end of the medullary tube shows on each side a lateral protrusion, the eye-bulb. At the anterior end of the body we can see the sense-plate and on each side the broad gill-plate. Lying in the sense-plate on each side is a deeply pigmented area which is the forecast of the "suckers," S. There is a depression in the middle line, in the centre of the sense-plate. This depression marks the mouth-depression, and indicates the point at which, later, the stomodæal invagination will take place. (See Fig. 20, F.)

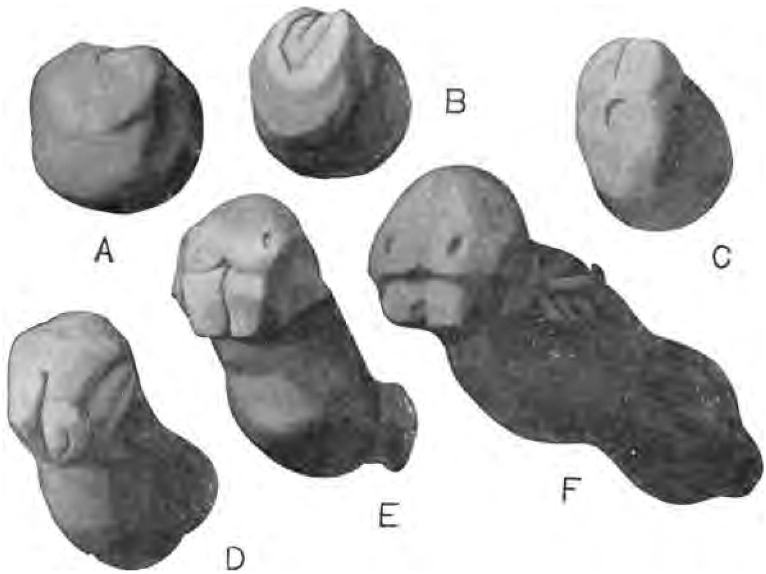

FIG. 22.—Development of embryo. Anterior view, showing sense-plate, nasal pits, stomodæum, and gills. (After Ziegler.)

A later stage is shown in Fig. 21, B. The relation of the parts is much as in the last figure. The anterior end of the medullary tube is larger than before, and the protuberances of the eye-evaginations are more apparent. In the gill-plate on each side appears a vertical depression and later another depression behind it, GS, GS′. These depressions mark the external gill-slits. The anus has shifted to a more ventral position. The suckers have each elongated ventrally, and have fused into a pigmented V-shaped figure. The outer medullary plates take no part in the rolling in to form the medullary tube, but flatten out and seem to disappear.

Ziegler ('92) has made several excellent figures of living embryos of Rana temporaria (Figs. 22, 23). The first of these shows young embryos as seen from in front, so that the sense-plate is turned toward the observer (Fig. 22). A longitudinal groove appears in the middle of the sense-plate, and subsequently a transverse groove develops across the sense-plate (Fig. 22, D, E). The depression that later forms the mouth lies at the crossing-point of the longitudinal and trans-

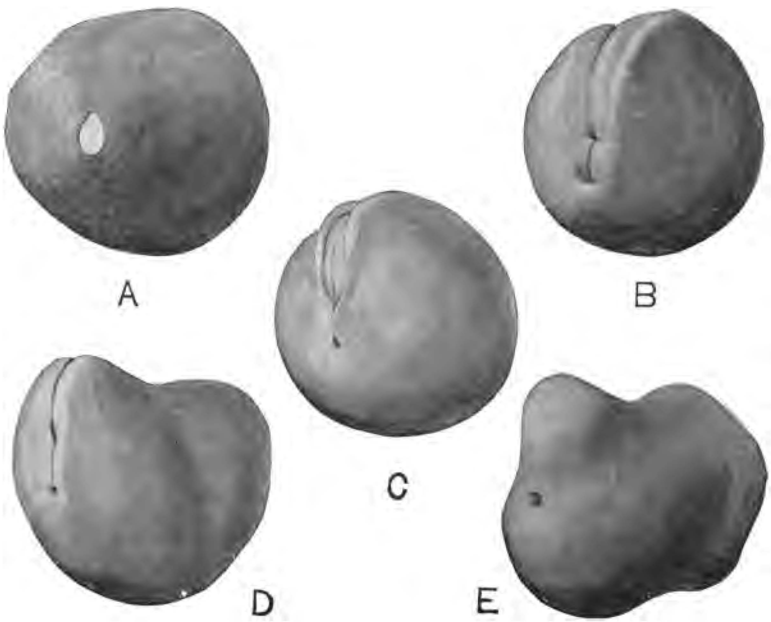

FIG. 23. — Development of embryo, showing closure of blastopore and formation of anus. (After Ziegler.)

verse grooves. There is present on each side above the mouth a thickened ridge that forms the superior maxillary process. Below and behind the mouth a pair of ridges appear that meet in the middle line. These are the sub-maxillary processes which later form the lower jaw. A pair of depressions of the surface ectoderm *below* the mouth-area mark very early the beginning of the "suckers," or adhesive glands (Fig. 22, D). The nasal pits appear above the mouth (Fig. 22, F).

The outlines of the three brain-vesicles can also be faintly seen in surface view. A pair of swellings on each side of the fore-brain shows the position of the eye-evaginations (Fig. 22, D). In the pharyngeal region there first appears on each side a vertical ridge, and later another ridge parallel to and behind the first (Fig. 22, D, E). On these ridges gills appear as protrusions of the surface, and later a third ridge and gill are formed behind and somewhat beneath the others. Some time after hatching, the *gill-slits* break through to the exterior between the ridges or gill-arches, and at about the same time, the mouth breaks through into the cavity of the pharynx.

In the head-region the beginnings of some of the spinal ganglia may be seen, and a series of mesodermal blocks also appear and may be dimly seen from the outer surface. These structures do not, however, appear as distinctly in surface views of the embryo of Rana temporaria as they do in the embryos of some other species.

Soon after the nerve-tube has closed, the dorso-posterior end of the body begins to extend backwards to form the tail (Fig. 23, D, E). The anal opening lies just behind and ventral to this region of posterior growth. The anus seems to shift to a more ventral position during the elongation of the tail. At first the tail is a thick outgrowth of the posterior end of the body, but as it grows longer it flattens from side to side, and in later stages a thin fan-like border or fin develops on its upper and lower margin (Fig. 38).