CHAPTER VII

THE PRODUCTION OF ABNORMAL EMBRYOS WITH SPINA BIFIDA

EMBRYOS of the frog are occasionally found that differ greatly from normal embryos. Roux, in 1888, first described one of these embryos and showed that a knowledge of its structure and method of development helped very much toward an understanding of the processes that take place in the

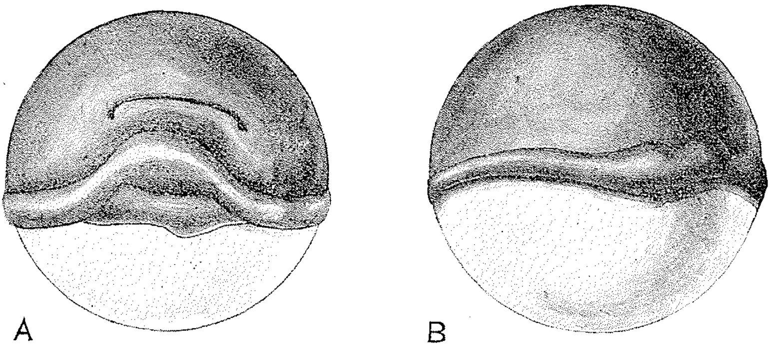

FIG. 27.—Two embryos formed as rings around equator of egg. A. Seen from in front (produced in salt solution). (Morgan.) B. Seen from side. (After Roux.)

normal development. An embryo described by Roux is shown in Fig. 27, B. Around the equator of the egg along the zone between the white and black hemispheres is a thickened ridge. A careful examination shows that this ridge is not uniform in thickness, but is bilateral in form. Each half is somewhat thickened at one end, and resembles half of the medullary plate of the normal embryo. Cross-sections (Fig. 29, B) show that these ridges around the equator of the egg are the two halves of the medullary plate. Instead, however, of being in close contact, the two half-plates are separated in the middle by the diameter of the egg, but at the anterior and posterior ends the half-plates unite to form the ring. In section, a cord of cells, the notochord, is found beneath each half of the medullary fold; and between the yolk-cells and the ectoderm there is also found a sheet of tissue representing the mesoderm. Hertwig, in 1892, described a large number of these embryos. One is shown in surface view as seen from the white pole, in Fig. 28, A. The embryo is at a later stage of development than that described above. The exposed white yolk, turned toward the observer,

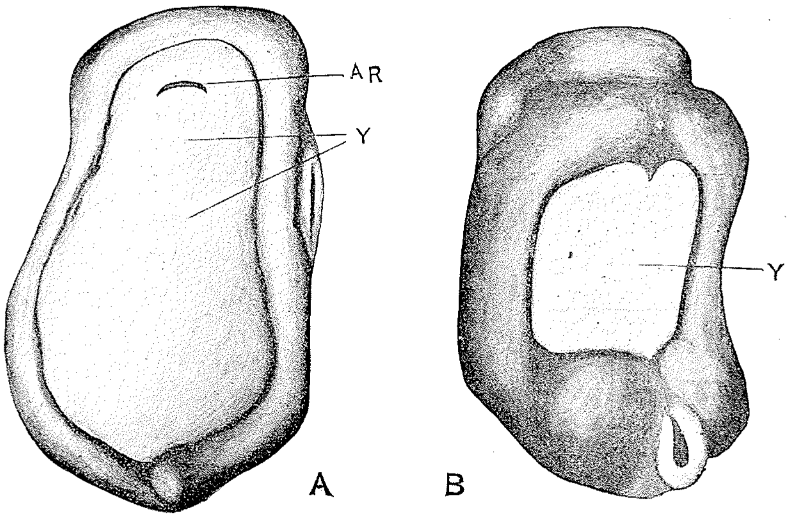

FIG. 28.—Two "spina-bifida" embryos. (After Hertwig.) A. Earlier, B. older stage (different embryos).

is surrounded by a groove, and outside of the groove there is a bounding darker ridge. In the anterior portion of the white is seen a crescent-shaped depression. A cross-section through the middle of the body of an embryo similar to the last is shown in Fig. 29, A. The exposed yolk is seen at Y. On each side of this there is a depression, and beyond the depression a thickened ridge composed of ectoderm cells. Each ridge passes over on its outer side into the ectoderm that covers all the lower part of the embryo. Even in their present stage of development the ridges are clearly seen to be the widely separated halves of the medullary plate. Beneath each half of the medullary plate there is a cross-section of the notochord, and between the yolk-cells, in the centre of the section, and the ectoderm covering the lower surface, there is a thick sheet of cells representing the mesoderm.

A longitudinal (sagittal) section of the embryo drawn in Fig. 28, A, is shown in Fig. 29, C. The large exposure of yolk-cells (Y) in the upper part of the figure is very conspicuous. A deep and narrow depression, bounded for the most part by a distinct layer of yolk-cells, is found near the anterior end. This depression corresponds to the crescent-shaped opening seen in surface view, and is supposed to correspond to a part of the archenteron of the normal embryo.[1] Ectoderm covers the lower (ventral) surface of this section, and at one point the cells are thickened to form the adhesive glands of the larva. At the posterior end of the embryo a small depression is present, and, as later development shows, this corresponds to the posterior portion of the archenteron of a normal embryo.

Hertwig found that if male and female frogs of certain species be separated and kept apart for several weeks, and the eggs then be artificially fertilized, an abnormal segmentation follows, and, although many of the eggs die, among those that live a large number show this condition of spina bifida.

In 1893 I made a series of experiments attempting to produce artificially embryos showing spina bifida, and found that they could be made by two entirely different methods. If the segmented egg, before the blastopore-lips appear, be placed in water to which .6 per cent. of salt (NaCl) has been added, the later development is modified. The dorsal lip of the blastopore appears in its normal position but does not continue to extend over the white hemisphere. The corners of the lips gradually extend around the equator of the egg. A sharp line or depression separates the black and white hemispheres, and on the black side of the depression a circular ridge appears, which marks the beginning of the medullary ring (Fig. 27, A).

Similar embryos may also be produced if the dorsal lip of

[1] Possibly it represents in part the liver-diverticulum.

the blastopore is injured with a needle at the moment of its appearance, or if the yolk-mass in front of the dorsal lip is injured so that the yolk protrudes from the general rounded surface of the egg. The blastopore is thus prevented from extending backward, and its material differentiates, *in situ*, along the equatorial line. The lateral lips tend to approach the middle line and to fuse, but the medullary folds may appear before the fusion has taken place. There is thus pro-

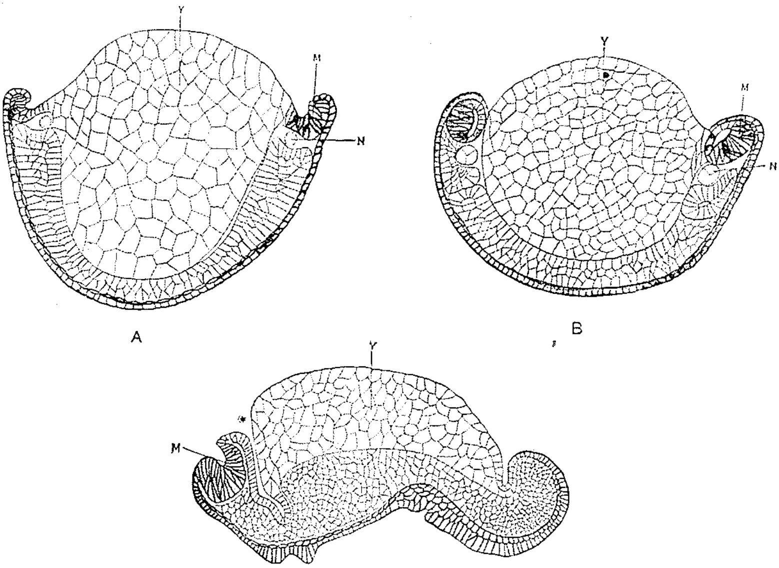

Fig. 29.—Cross (A, B) and longitudinal (C) sections through an embryo with spina bifida. (After Hertwig.) M. Half medullary plate. N. Half notochord. Y. Yolk.

duced an embryo with an exposure of yolk in the mid-dorsal line. The exposure is more or less extensive, according to the extent of fusion anteriorly of the blastopore, and to the extent of fusion forwards of the lateral and ventral lips.

These embryos with spina bifida show that the material for the mid-dorsal surface of the embryos appears first as a ring around the equator of the egg or a little below the equator. If this material is prevented from reaching the mid-dorsal surface, *it differentiates in situ.* Hence the production of a ring-like medullary plate and a double notochord.

It is important to know definitely the origin of the material that forms the equatorial ring. We have seen that the ring appears at the same time that the blastopore-lips extend around the equator of the egg. Does this material also extend out laterally from the dorsal lip of the blastopore along the sides, or is the material already present as a circular ring of tissue, from which the lips of the blastopore differentiate? A study of the normal embryo combined with experiments gives, I believe, a conclusive answer to these questions. In the first place, if the *dorsal lip* be entirely destroyed, so that it cannot advance, nevertheless the lateral lips still appear and extend backward. If a point of the surface be injured just in front of one (or both) of the advancing *corners of the dorso-lateral lips*, the advance of the latter would be stopped if an actual transfer of material were taking place; nevertheless, on the posterior side of the point of injury, a depression of the surface, marking the blastoporic rim, appears, and continues to extend backward. The same thing happens if injuries be made at two consecutive points in the direction of extension of the lateral lip. Now if material were actually transferred backward from the dorsal lip and around the equator of the egg, its movement would be stopped when the dorsal lip was seriously injured, so that the lateral lips of the blastopore, and, later, the medullary folds, would not appear, or else their appearance would be delayed. Further, if there were, in reality, any such transfer backward of material around the equator, its progress would be stopped when the material reached the points of injury made along the line of the lateral lip. On the contrary, the appearance of the lateral lips, after the destruction of the dorsal lip, takes place as though no hindrance were present.

The experiments point clearly to the conclusion that there is no backward transfer of building material, but that the material for the dorsal surface is already present as a ring around or near the equator of the egg.

If the normal embryo be studied by means of sections at the period of the extension of the lateral lips of the blastopore, the material of the ring is found to be already present in the region into which the lateral lips extend. The evidence from these various sources proves *that the production of the embryos* *showing spina bifida is owing to the differentiation in situ of cells that in the normal embryo are first carried to the dorsal surface before they differentiate into their definitive organs.*

Roux first pointed out that the embryo described by him showed that the material for the two sides of the embryo is laid down in a ring, and that by the growing together (concrescence) of this ring along the mid-dorsal line of the embryo, the two halves of the body are brought together. The same method of formation of the embryo by concrescence has been described as taking place in other vertebrate embryos, and certain writers have even affirmed that this is the method by which all embryos of vertebrates are formed. In the main, Roux's conclusion for the frog seems to be correct,[1] but in one respect not an unimportant exception must be taken to his statement. If the material be laid down as a ring of tissue around the equator, and if, by its coming together (apposition), the two halves of the embryo result, it follows that the embryo should be at least as long as one semicircle of the surface of the egg. Further, we have seen that the anterior end of the medullary plate lies somewhat above the point of appearance of the dorsal lip of the blastopore, so that the embryo would be, on Roux's supposition, even longer than a semicircle. But if we measure the medullary plate of the embryo at the time of its *first appearance*, we find that in length it is only about one-third of the length of the circumference of the egg. It follows, then, that as the material comes to the mid-dorsal line in the normal embryo, it must also become more concentrated, so that the length of the medullary plate is less than the length of the material of its halves. *There is an accrescence or concentration of material combined with a concrescence or union of material from the two sides.*

[1] Although Roux did not foresee the possibility that material might grow around the equator from the dorsal lip of the blastopore, my own experiments show, I think, that such a transfer does not take place.