The Relation Between Normal and Abnormal Development of the Embryo of the Frog:

IX. As Determined by Insufficient Aeration.

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With 18 figures in text.

The retarded development of eggs that lie in the middle of the bunch, or, if the bunch is confined in a flat dish, in the middle of the lower surface of the bunch, must have been observed by everyone who has kept frog's eggs in a confined space. The delay is caused probably in part by insufficient aeration, but whether because of lack of oxygen, or because of an excess of carbon dioxide or of some other substance excreted has not been sufficiently determined. Several conditions may often be involved, for, it has been shown by GODLEWSKI that carbon dioxide acts injuriously on the eggs of the frog, and SAMMASSA, SCHULTZE, and GODLEWSKI have shown that an insufficient supply of oxygen also causes the egg to develop abnormally.

In the following pages I shall describe some abnormal embryos of *Rana palustris* that were taken from the under side of bunches of eggs developing in flat dishes. The embryos at the top of the bunch had begun to hatch when the oldest of these abnormal forms were preserved.

The most common feature, shown by all of the eggs that result from insufficient aeration, is that the yolk fails to be drawn under the black cap and the latter is generally sharply marked off from the yolk, often by an inturned edge. Ring-embryos are not infrequently found in lots of this sort, and some forms that originated in this way I have already described. I shall omit, therefore, an account of the ring-embryos and spina bifida forms in the present paper.

A not uncommon type is represented in text-figure 1 in which a dark hemisphere is separated from a white hemisphere by a narrower portion. A section through this embryo in the plane of the paper is shown in text-figure A. The black cap is made up of a thick layer of ectoderm. Around the sides it turns under to form a cavity. At the top of this cavity elongated cells are present that recall the

elongated cells at the top of the archenteric invagination in the normal gastrula. The interior cells above this cavity may possibly correspond to mesoderm cells. The rest of the egg is composed of yolk cells in the middle of which there is a large cavity that extends from the top to the bottom of the egg. This cavity has the appearance of being an enlargement of the segmentation cavity, but an unbroken series of stages would be necessary to establish this point. It should, however, be recalled in this connection that in the late stages of normal development, after the yolk has grown upwards around the inside of the segmentation cavity, the latter sinks down into the yolk, and may come to lie close to the floor of the archenteron, being separated from it by only a single line of cells that is often broken in preserving or in cutting the egg. The floor of the segmentation cavity is derived from the cells of the lower hemisphere, so that the extension downwards of the segmentation cavity in these abnormal embryos may not be so very different from the changes that take place in the normal. The flattening of many of the cells that border the central cavity is noticeable but the meaning of this is obscure.

An embryo similar in many ways to the last is shown in Fig. 2. The conditions are similar to the last, except for the much smaller central cavity, which is largely confined to the bottom of the egg, although there is a central core of loose cells through the middle of the embryo.

The next two embryos, Figs. 3 and 4, are much larger than the preceding ones. The enlargement is clearly associated with an enormous cavity in the interior of the egg, as shown in Figs. *C*, *D*. These eggs are so abnormal and so little developed that there is little hope of identifying the different parts. There is no sharp line between the dark and the light cells, but the deeper pigmentation of a band of cells near the equator may indicate the region of the blastoporic rim of less modified eggs¹).

Another embryo, externally much like the preceding, is shown in surface view in Fig. 5, and in section in Fig. *E*. In this embryo a large part of the yolk has been so seriously injured that it has failed to become cellulated (or if it divided in the early stages the planes of division have disappeared). On one side an archenteron

¹) I have found somewhat similar embryos from eggs kept in the cold, but here too the influence may have been due to lack of aeration rather than to the cold.

has developed, and here above the archenteron and around its top a few yolk cells are present. A large segmentation cavity occupies the top and middle of the yolk. It is probable that the dorsal lip of the blastopore has pushed backwards for a short distance over the yolk. A small amount of mesoderm also appears in the lip of the blastopore.

Fig. 6 shows the anterior end of an embryo lying apparently high up on the dark hemisphere. The longitudinal section Fig. *F* gives a more correct idea of the conditions. The section shows that half, at least, of the top is dead, and has lost much of its pigment. Hence the embryo appears to lie nearer the top of the egg than is really the case, since, with the loss of pigment on one side, the top is no longer so sharply defined. Practically all of the yolk is dead and vacuolated. It appears, therefore, that only one side of the egg has remained alive, and has formed the anterior end of the embryo; in part by a backgrowth over the dead yolk, which must have been brought about, no doubt, largely by the approach of the lateral lips to the middle line. The archenteron is a long slit with its anterior walls surrounded by the few yolk cells that are present. The roof of the remaining part is covered by small cells, which we must suppose to have been carried to this region by the coming together of the sides of the blastopore in the dorsal lip. As this occurred the posterior end extended over the yolk. Thus while the embryo appears, in surface view, to lie over the top of the egg, it occupies in reality one side. It may be admitted as a possibility that as the lateral lips approach the anterior end of the embryo may as a whole be shoved somewhat forward, so that as it forms the head-end may be carried to a position a little anterior to that at which it first appeared.

The next figure, Fig. 7, shows the anterior end of an embryo with the medullary plate in process of rolling in. At its posterior end the plate appears as though split into right and left halves between which the yolk appears on the surface as a V-shaped mass at the posterior end. A longitudinal median section, Fig. *G*, shows that this type of abnormality has been caused by injury to the yolk of the lower hemisphere, so that it fails to be carried under the dorsal and lateral lips of the blastopore. The latter have advanced only for a short distance at the sides. The section was a little oblique so that the medullary plate is not cut through its entire length. The ventral lip has failed completely to develop.

Figs. 1—9.

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The embryo drawn in Fig. 8 shows, in surface view, little more than a black and a white half, sharply separated by an inturned

Figs. *A—I*.

rim. In section, Fig. *H*, an imperfectly formed half-embryo is found along the black white edge. The medullary plate is scarcely developed;

a notochord lies below or near the edge, with an archenteric slit on its inner side. The other half of the egg is so much injured that it has failed to produce an embryo.

An embryo with an exposure of yolk at the side is shown in Fig. 9. The head is nearly normal and equally developed on both sides, but the middle and posterior regions of the embryo are incomplete on the side of the exposed yolk. A cross-section through this region is represented in Fig. *I*. The dorsal side appears more than a half structure, at least to the extent of having some mesoderm on the inner side of the notochord. The inturning of the archenteron, just outside of this small patch of mesoderm, shows that the region represents the blastoporic rim of the right side of the embryo. The mesoderm may be looked upon as having arisen as an extension backwards of the mesoderm of the right side from the more anterior regions of the embryo that are symmetrically developed, or it may be supposed to be due to the notochord developing a little inside of the edge, so that some of the mesoderm is left on the yolk side. Both interpretations, in the light of results of this sort in other cases, seems to me to be the more possible. The section shows also the other side of the exposed yolk (at the bottom of the figure), which theoretically corresponds to the missing half of the blastoporic rim. No development has taken place on this side, and it is due to this that the yolk has remained uncovered. It is worthy of notice, that here, as in other cases of this sort in which one blastoporic rim fails to develop, the developing side produces something more than half.

Summary.

1) The principal injury caused by insufficient aeration is to the yolk which failing to turn in interferes with the downgrowth of the material at the equator of the egg. Ring embryos and embryos with spina bifida are of frequent occurrence.

2) More extreme injury results when the earlier stages are affected producing embryos like those shown in Figs. 1 (*A*), 2 (*B*), 3 (*C*), 4 (*D*). In these the segmentation cavity has extended downwards into the yolk instead of outwards towards the sides.

3) In other cases the yolk may be actually so injured that it fails to segment, or, if segmented, the cells walls disappear and the yolk becomes vacuolated or dead. According to the extent of injury

to the yolk different types of embryos appear, some of which are shown in Figs. 5 (*E*), 6 (*F*), 7 (*G*). In most cases the injury involves equally all sides of the yolk so that a circular blastoporic rim develops Figs. 5—7.

In other cases, Figs. 8 and 9, one side may be more injured than another so that it develops less. This difference may be due to one side of the egg being in a better position to become aerated than the other.

Zusammenfassung.

1) Die Schädigung, welche ungenügende Durchlüftung prinzipiell hervorbringt, geht den Dotter an, welcher aus Mangel an Einwärtsbewegung das Herabwachsen des Materials vom Eiäquator stört. Ringembryonen und solche mit *Spina bifida* kommen häufig vor.

2) Extremere Schädigung ergibt sich, wenn die früheren Stadien betroffen werden; es entstehen dann Embryonen ähnlich denen in Fig. 1 (*A*), 2 (*B*), 3 (*C*), 4 (*D*). Bei diesen hat sich die Furchungshöhle nach unten in den Dotter ausgedehnt, anstatt nach außen, nach den Seiten zu.

3) In andern Fällen kann der Dotter tatsächlich so verletzt sein, daß seine Furchung ausfällt, oder daß im Falle der Furchung die Zellwände verschwinden und der Dotter vacuolisiert wird oder abstirbt. Entsprechend der Ausdehnung der Dotterschädigung erscheinen verschiedene Embryotypen, von denen einige in Fig. 5 (*E*), 6 (*F*), 7 (*G*) dargestellt sind. Dabei bringt in den meisten Fällen die Schädigung den Dotter auf allen Seiten in der Entwicklung zurück, so daß sich eine zirkuläre Blastoporusspalte entwickelt (Fig. 5—7).

In andern Fällen (Fig. 8 und 9) kann eine Seite mehr geschädigt sein als eine andre, so daß die letztere sich besser entwickelt. Diese Verschiedenheit kann von einer günstigeren Lage der einen Eiseite mit bezug auf die Durchlüftung bedingt sein.