# THE EMBRYOLOGY OF CRYPTOBRANCHUS ALLEGHENIENSIS, INCLUDING COMPARISONS WITH SOME OTHER VERTEBRATES

## II. GENERAL EMBRYONIC AND LARVAL DEVELOPMENT, WITH SPECIAL REFERENCE TO EXTERNAL FEATURES[1]

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TWO HUNDRED AND TWENTY-THREE FIGURES (EIGHT PLATES)

CONTENTS



## VI. INTRODUCTION

The present contribution is one of a series of papers on the embryology of Cryptobranchus, of which Part I, dealing with the breeding habits, ovogenesis, maturation and fertilization, has already been published (Smith '12). In the preparation of

[1] Part I was published in the Journal of Morphology, vol. 23, no. 1, March, 1912.

this paper I am greatly indebted to Prof. Bashford Dean, under whose guidance it has been brought to completion.

During the past seven years the collection of an abundance of material has enabled me to preserve an ample supply in every stage of development. At least fifteen thousand embryos have been secured from nests, and nearly as many more have been obtained by artificial fertilization.

For convenience in description, the embryonic and larval history has been divided into stages, based chiefly on external characters. In making the division, the usual difficulty has been encountered, that the rate of development of each structure varies more or less in different embryos. In determining what shall constitute the interval between stages, the guiding principle has been to establish stages only so far apart that individual variations in the rate of development of the most important characteristics selected as criteria for classification shall not overlap. For purposes of more intensive study, each stage may be divided into tenths; this device is useful in following any single character or set of characters. Since development is a continuous process, the importance of studying a close series cannot be too strongly emphasized.

As an aid to obtaining the exact sequence of events, stress has been laid on the study of a series of stages preserved at short intervals, from a single lot of eggs fertilized at the same time. Every period of the embryonic development has been covered repeatedly in this way, an entire spawning of eggs being sometimes used in the study of three or four stages as distinguished in this account. Thus not only a close series, but a large number of embryos in each stage, representing several different spawnings, have been studied, so that the typical course of development could readily be distinguished from variations or abnormalities.

Moreover, the entire embryonic and larval history has been carefully followed in living material, repeatedly and for the most part with embryos freshly collected. The study of living material is of especial importance in the late cleavage and gastrula stages of Cryptobranchus; for in these stages the translucent condition of parts of the unpigmented embryo enables one to

gain a fair idea of what is going on inside. The ciliation of the ectoderm of the late embryo, and some features of the circulatory system, are best studied in living material.

An accurate and complete time record (Section X) of the course of development has been obtained by comparison of many different records of material kept alive during long periods of time; these results were checked by observing the rate of development of material freshly collected. One lot of embryos, collected in the fall of 1906 at the time of the closure of the neural folds, were kept alive in the Zoological Laboratory of the University of Michigan throughout the entire larval period, and their metamorphosis was observed at the end of the second year after fertilization. Specimens were preserved at intervals; shortly after metamorphosis the half-dozen remaining individuals died from causes unknown. Another lot of embryos collected in an advanced gastrula stage in the fall of 1910, were kept alive and in good condition in the Zoological Laboratory of the University of Wisconsin until May, 1911, when the last ones were preserved.

The study of external and internal structure has gone hand in hand, except for the post-gastrula stages; here, doubtful points in the interpretation of the external structure have in most cases been investigated by reference to serial sections.

In preparing the illustrations, composite or ideal figures have been avoided. Each drawing, unless otherwise specifically stated, is a faithful representation of an individual embryo; a sufficient variety of figures has been given to illustrate the most important deviations from the condition regarded as typical. All the drawings are the work of the author except figures 268 to 276 which were drawn by Prof. Bashford Dean and with his generous permission are here published for the first time; figure 203 which was kindly contributed by Dr. L. Hussakoff of the American Museum of Natural History; and figures 277 to 279 which were drawn by Miss Hedge of Columbia University.

The histological technique employed has already been given in Part I; it remains to record the methods used in photography. For embryonic stages, fixation in Solution B (see Part I) followed

by preservation in formalin, gives the best photographic results. While being photographed, the objects were immersed in water or formalin. Living larvae were anesthetized with chloretone. In all cases the exposure was made by daylight.

All the photographs with a magnification of × 4 were made with a Bausch and Lomb Zeiss Tessar 72 mm. lens, fitted to a long bellows Pony Premo No. 6 camera. The camera was fastened in an erect position by means of an improvised wooden frame. Figures 262, 264, 265 and 266 were taken with a Zeiss Unar Lens; figures 263 and 267 with a Zeiss Apochromatic Planar. Seed's Non-halation plates were used throughout; they were developed with Adurol.

All the negatives are the work of the author except figure 266 which was made by Miss Frances J. Dunbar of the University of Michigan.

The photographs are untouched, except in a very few cases for the purpose of correcting slight defects in the negatives.

## VII. CLEAVAGE

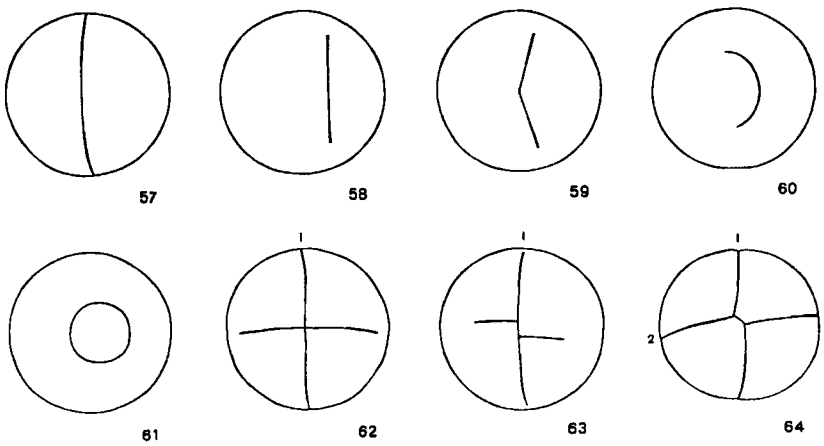
### *A. Description of cleavage by stages*

*Stage 1: (figs. 57 to 61 and 204).* This stage is characterized by the presence of the first cleavage furrow only. The germinal area reaches nearly to the equator.

In artifically fertilized eggs the first cleavage furrow ordinarily appears about twenty-four hours after fertilization; the time may vary from eighteen to twenty-eight hours. The furrow begins as a pit, usually at the animal pole; it lengthens rapidly at first, then more slowly as it invades the regions of the egg more heavily laden with yolk. After the first cleavage furrow is well established, it becomes narrow in its middle portion, while still broad at the ends. The first cleavage furrow of the typical form becomes superficially complete in Stage 3 (third cleavage).

In the typical condition, the first cleavage furrow passes through the animal pole, and the division is equal (figs. 57 and 204). Variations from this condition occur. To test the amount of

variation, sixty eggs from a single spawning were examined in the first cleavage stage. In forty-eight cases the condition was of the typical character described above. In seven cases the first cleavage furrow was straight, but the cleavage unequal; figure 58 represents the extreme of this condition. In three cases, the first cleavage furrow passed through the animal pole, but its halves met at this pole to form an obtuse angle (fig. 59).




Figs. 57 to 64 Types of first and second cleavage of Cryptobranchus allegheniensis. × 3½.

Fig. 57 The first cleavage furrow extends just to the equator, a little further than is usual before the appearance of the second furrow.

Fig. 62 The first cleavage furrow extends a little below the equator.

Fig. 64 The first cleavage furrow extends a little below the equator; the second furrow extends just to the equator.

In two cases the first cleavage furrow formed a semicircle about the animal pole (fig. 60). In two cases from different spawnings, neither of which furnished the material for the above data, circular first cleavage (fig. 61) was found; in each case the animal pole was excentrically situated within the area bounded by the cleavage furrow.

Goodale ('11) reports a case of circular first cleavage in Spelerpes; the egg gave rise to a normal embryo. According to Eycleshymer ('04), in Necturus the cytoplasm is always unequally divided by the first cleavage, giving rise to blastomeres which in many cases are decidedly unequal.

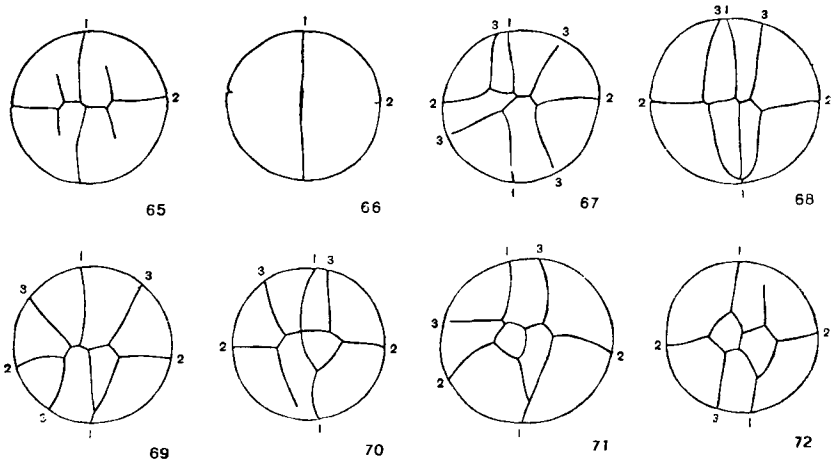
*Stage 2: (figs. 62 to 64 and 205).* The second cleavage furrow makes its appearance about six hours after the first, which by this time extends nearly to the equator of the egg. In the stage represented in figure 205, the first cleavage furrow has just reached the equator. The second cleavage furrow usually becomes superficially complete in Stage 4 (fourth cleavage), quite uniformly meeting the first cleavage furrow at right angles at the vegetal pole.

The earliest indication of the second cleavage furrow is usually a roughness in the region of the animal pole where the second groove is to intersect the first. The occurrence of 'Faltenkranzen' —a quivering of the surface with the formation of fine radiating or parallel wrinkles, which extend outward from the cleavage furrow—is quite marked at the time of the initiation of the second cleavage furrow. The cause of the formation of similar wrinkles in the frog's egg has been investigated by Charles B. Wilson ('96). For some time after its first appearance the second furrow is much broader, though of course shallower, than the first.

The second cleavage furrows usually depart from the same point as the first furrow, and proceed vertically, forming a single straight line at right angles to the first furrow (figs. 62 and 205). Occasionally the points of departure of the second furrows do not coincide, as shown in figures 63 and 64. The condition shown in figure 63 is rarely observed, and is transitional to that shown in figure 64, which is quite frequent. That portion of the first cleavage furrow lying between the points of departure of the second furrows may be called the polar furrow. As shown by the individual histories of a large number of eggs, in all cases in which a polar furrow is present the points of departure of the second cleavage furrows are separate from the beginning; the polar furrow at first exists as a part of the straight first cleavage furrow, but later becomes oblique through the shifting of cells. In no case in which the second cleavage furrows have their origin from the same point in the first cleavage furrow, has there ever been observed any shifting of cells during this or the following stage, of such a nature as to produce a polar furrow.

Comparison with the second cleavage of Necturus as figured by Eycleshymer ('04), Eycleshymer and Wilson ('10), and in some unpublished drawings of the living egg by Prof. Bashford Dean, leads to the conclusion that in Necturus there is much greater irregularity in the second cleavage than in Cryptobranchus.

*Stage 3: (figs. 65 to 72 and 206).* The third cleavage furrows appear about five hours after the beginning of the second; hence the interval is shorter than that between the first and second. At the time when the third furrows begin, the first furrow has usually reached or passed the equator.




Figs. 65 to 72 Types of third cleavage of Crytobranchus allegheniensis. All the figures are of the upper hemisphere except figure 66 which represents the lower hemisphere of the egg shown in figure 65. In no case does any cleavage furrow except the first reach the lower pole. All the figures are camera drawings of preserved material. × 3½..

As the cleavage furrows invade the more heavily yolk-laden lower hemisphere they become comparatively faint except at their extreme lower ends where they broaden out. During this stage the second and third cleavage furrows are in general broader than the first.

At the stage represented in figure 65, when the third furrows are well established but extend only a short distance from their

respective points of origin, the first cleavage furrow has reached the lower pole where its ends unite. The first cleavage furrow thus becomes superficially complete, thereby establishing the holoblastic character of the egg. That there is a strong meroblastic tendency is already apparent. In the region of the vegetal pole the first cleavage furrow is at first broad, but it later becomes narrow and faint.

With very few exceptions, the third cleavage furrows depart from the *second* furrow, at some little distance from its point of intersection with the first. In a previous paper (Smith '06) it was erroneously stated that the third cleavage furrows usually depart from the first furrow.

The third cleavage furrows ordinarily begin as two pits in the second furrow, equidistant from its point of intersection at the animal pole. From these two pits the third cleavage furrows ordinarily proceed in an approximately vertical direction (fig. 65), and do not become complete until a later stage (Stage 5).

From the time of the earliest appearance of the third cleavage furrows, the distances from the first cleavage furrow to their points of departure from the second remain unaltered; but the second cleavage furrow, originally straight, often becomes drawn into a zig-zag line, as shown in the figures.

As will be seen from a study of later stages, the third cleavage furrows rarely reach the vegetal pole, but as a rule extend obliquely in the lower hemisphere to join the *first* furrow at some distance from the lower pole (figs. 84 and 91 to 96). Hence the general statement may be made that the third cleavage furrows are intermediate between a true meridional and a true latitudinal cleavage but approach more nearly to the former type.

In the typical condition, the cleavage pattern has now lost its strictly radial, and acquired a biradial symmetry. I have purposely avoided the use of the word *bilateral* in this connection, not only because it does not fit the case so well as the word *biradial*, but in order to avoid the inference that the condition has anything to do with the bilateral symmetry of the future embryo. As will be seen by consulting the figures, this biradial condition of the cleavage pattern persists in the lower hemisphere through-

out the late cleavage stages, and in some eggs enables one to identify the early cleavage furrows even after the beginning of gastrulation.

Deviations from the type in Stage 3 show a series of conditions connecting the typical one with a true latitudinal third cleavage. In such cases the third cleavage furrow proceeds more obliquely, and at an earlier stage joins the first nearer the animal pole (figs. 68 to 72). In some cases one or more of the third cleavage furrows are truly latitudinal (figs. 70 to 72).

Rare cases occur in which a third cleavage furrow originates at the animal pole, or from a first cleavage furrow (see fig. 67 for an example of the latter case); occasionally, a third cleavage furrow may reach the vegetal pole, or unite with a second cleavage furrow near the vegetal pole (figs. 94 and 95).

In comparing the third cleavage pattern of Cryptobranchus with that of other forms, one of the most obvious generalizations brought out is that a vertical third cleavage is characteristic of heavily yolk-laden and highly telolecithal eggs: e.g., the squid (Watase '91); Amia (Dean '96, Whitman and Eycleshymer '97); Lepidosteus (Dean '95, Eycleshymer '99); Acipenser (Dean '95); Ctenolabrus (Agassiz and Whitman '84); Serranus (H. V. Wilson '91); Ceratodus (Semon '00 and '01); Lepidosiren (Kerr '00 and '09); Cryptobranchus japonicus (deBussy '04 and '05); and the pigeon (Blount '07). But the rule is not absolute; concerning the third cleavage of Necturus, Eycleshymer ('04) says: "In most cases the cleavage grooves are irregularly formed and it might be said that the variations are so numerous and so diverse that a special description must be written for each egg." From this statement and an inspection of his figures (see also Eycleshymer and Wilson '10), it appears that a type cannot be recognized for the third cleavage of this egg; that the irregularity is greater than in the case of Cryptobranchus and that there is a more marked tendency for the third cleavage furrows to come in latitudinally. My material for the very early cleavage stages of Necturus is too scanty to enable me to form any conclusion based on direct observations, but some unpublished figures of the early cleavage of Necturus drawn from the living egg by

Prof. Bashford Dean give confirmatory evidence of the irregularity of the third cleavage furrows. In Desmognathus the third cleavage furrows were vertical and regular in the few eggs studied by Wilder ('04); the third furrows depart from an earlier cleavage furrow at some distance from the animal pole. But Hilton ('04 and '09), who examined a considerable number of eggs of Desmognathus in this stage, states that this regular and vertical form of cleavage occurred in only two or three eggs; in the others the third cleavage was irregular. In Diemyctylus (Jordan '93) there is still greater irregularity in the third cleavage than is recorded for Necturus or Desmognathus: "With the completion of the second furrow all consistent regularity is at an end."

In eggs less heavily yolk-laden, as in Amblystoma (Eycleshymer '95) and the frog, the third cleavage is latitudinal.

Especially interesting from a comparative point of view are Budgett's observations on the cleavage of the crossopterygian Polypterus as given by Kerr ('07). "From Budgett's pen and ink sketch . . . . . we can see that the segmentation is at first characterized by its almost absolutely equal character. We may infer with considerable certainty that the two meridional furrows are succeeded by a latitudinal one which is practically equatorial." The egg of Polypterus is small, having a diameter of a little over one millimeter.

In urodeles we find a condition intermediate between the vertical third cleavage characteristic of the fishes generally, and the latitudinal third cleavage of the anura. In Cryptobranchus the vertical type prevails; in Desmognathus, Necturus and Diemyctylus there is increasing irregularity; in Amblystoma the third cleavage is latitudinal. The possible phylogenetic significance of the cleavage of Crytpobranchus will be considered later.

As a rather general rule, in eggs in which the third cleavage is usually vertical, the third furrows depart from the second rather than from the first or from the animal pole. As has already been seen in the case of Cryptobranchus allegheniensis, this rule is by no means absolute; but in general it applies also to the squid, and to the teleosts (e.g., Ctenolabrus, Serranus). DeBussy ('04 and '05) has described the cleavage stages of

Cryptobranchus japonicus; his material was meager and lacked first and second cleavage stages. He states ('05) that in the five eggs examined in the third cleavage stage, all the third furrows are approximately meridional. His figures ('04) represent the third furrows departing most frequently from the first cleavage furrow, sometimes from the second, sometimes from the animal pole. In the urodele Hynobius (Kunitomo '10) the cleavage pattern in this stage resembles that of Cryptobranchus alleghe-niensis, except that the third furrows do not so often depart from the second furrow. In Amia, Whitman and Eycleshymer ('97) describe the third cleavage furrow as follows:

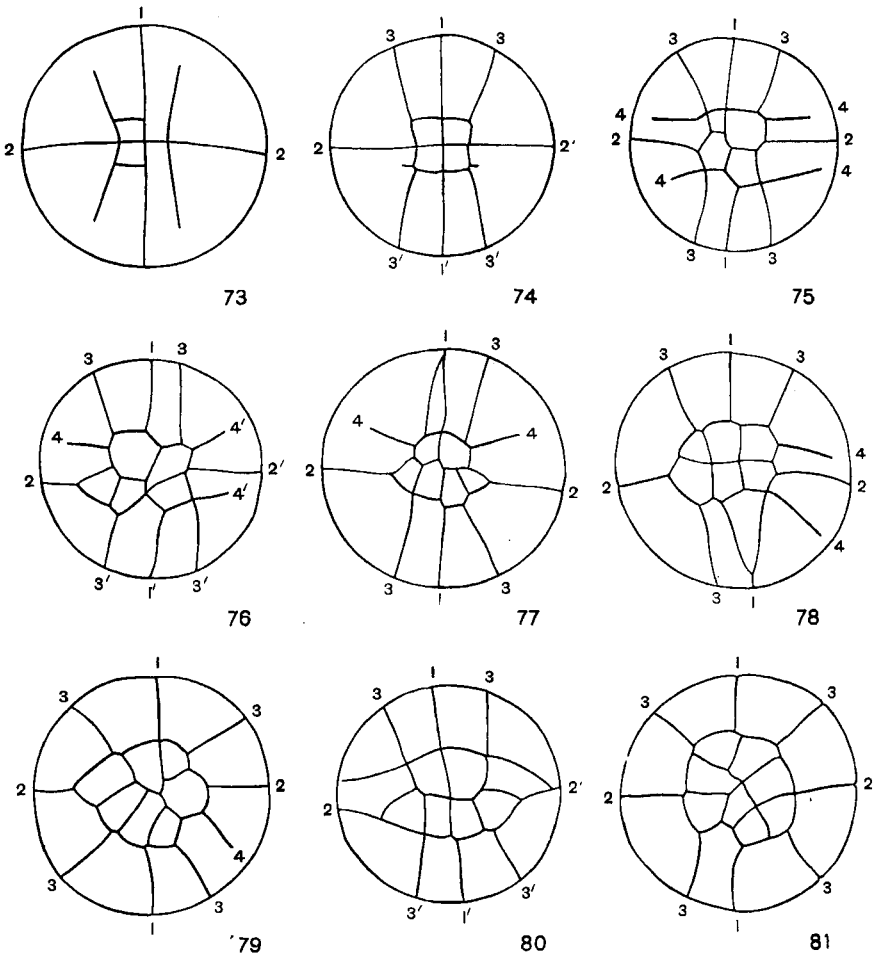
> In the majority of cases they are vertical . . . . They generally all depart from one or the other of the first two meridionals, thus giving rise to a distinct bilateral appearance . . . . It oftens occurs that one or more of the set depart from the first meridional, while the rest depart from the second, or *vice versa*.

*Stage 4: (figs. 73 to 84 and 207 to 209).* This stage is characterized by the appearance of the fourth cleavage furrows, giving, when complete, sixteen cells. As will appear from the following observations, the number of micromeres is not constant, but varies from four to eight.

The fourth cleavage furrows appear about four hours after the beginning of the third, and about thirty-nine hours after fertilization. Ordinarily they begin as two grooves, cutting the *first* cleavage furrow at right angles, on each side of the second and a short distance from it (figs. 73 and 74). Thus in position and direction the fourth cleavage furrows alternate with the third, which cut the *second* at approximately right angles. The fourth cleavage is the first one to cut off micromeres from macro-meres, and the division is very unequal.

In a given lot of eggs the sixteen-cell stage is reached quite uniformly at the same time, but with so much variation in the direction of the fourth cleavage furrows that at first sight no uniformity is recognizable. By the study of a large number of eggs the following generalizations are established:

(a) In each quadrant, one micromere is cut off between the first and second cleavage furrows, giving in each egg a minimum of four micromeres (fig. 74).




Figs. 73 to 81 Fourth cleavage of Cryptobranchus allegheniensis, × 4⅔. All the figures are of the upper hemisphere. Figure 73 and 74 are drawn from the living egg; the others are camera drawings from preserved material. Figures 73 and 74 represent early stages of fourth cleavage; figure 80 is from the same egg shown in figure 74, representing the condition three hours later. Figure 81 is drawn from the egg photographed for figure 209.

(b) From the third cleavage furrows the remaining four parts of the fourth cleavage furrows may continue latitudinally, forming a complete circle or oval enclosing eight micromeres (fig. 81); or one or more of these four parts may continue approx-

imately parallel to the second cleavage furrow, extending vertically and increasing the number of macromeres instead of cutting off micromeres (figs. 75 to 80). Thus while the total number of cells is always sixteen, the number of micromeres varies from four to eight.

We thus get as one extreme type an approximately latitudinal fourth cleavage furrow; as the other extreme a fourth cleavage furrow divided into two separate grooves, one on each side of the second furrow and approximately parallel to it and to each other. Between these two extremes we find examples of all possible intermediate conditions.

With regard to the manner of fourth cleavage, eggs of this stage may be classified into five types, depending on the number of micromeres present. For the purpose of such a classification, irregularities in the third cleavage must be allowed for: in cases where the third cleavage has come in diagonally or latitudinally to cut off a small cell, such a cell is divided by the fourth cleavage into two small cells, of which only the one nearer the animal pole is to be counted as a micromere (fig. 77).

To determine the mode, twenty-five eggs were examined in the sixteen-cell stage, and the results tabulated as follows:

| Number of micromeres | 8 | 7 | 6 | 5 | 4 |
|---|---|---|---|---|---|
| Number of cases | 4 | 7 | 8 | 3 | 3 |

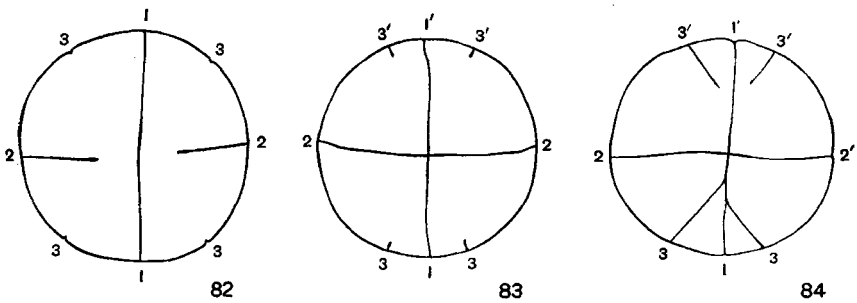
The table shows that the most frequent manner of cleavage is intermediate between the two extremes described.

In the majority of cases the micromeres are arranged with considerable regularity in two parallel rows, separated by the second cleavage furrow (see especially figs. 78 and 80). This is the necessary result of the biradial symmetry instituted by the normal mode of third cleavage, providing there is no extensive shifting of the micromeres. The condition reminds one of the cleavage pattern of the corresponding stage of the teleost egg.

Through a shifting of the micromeres, the biradial symmetry of the cleavage pattern of the blastodisc is usually interfered with (figs. 75 to 81). In this region, the first and second cleav-

age furrows become irregular and broken to an extent never observed in earlier stages. Outside of the region of the micromeres, the biradial pattern of cleavage is retained.

In this as in other cleavage stages the most recent furrows, and especially the most recent portions of such furrows, are in general quite noticeably the widest. This fact once established may be made use of in connection with other evidence to identify cleavage furrows. The broadening of the ends of the vertical furrows as they invade the lower hemisphere is a fairly constant feature of the cleavage; as shown by unpublished drawings from living material by Prof. Bashford Dean, it is also well expressed in the eggs of Necturus.




Figs 82 to 84 Lower hemispheres of fourth cleavage stages of Cryptobranchus allegheniensis. All the figures are camera drawings of preserved material. × 4⅔.

Fig. 82 Lower hemisphere of the egg shown in figure 208.

Fig. 83 Lower hemisphere of the egg shown in figure 76. This figure would serve equally well to represent the lower hemisphere of the egg drawn for figure 75.

Fig. 84 Lower hemisphere of the egg represented in figure 80.

In the majority of eggs of this stage, the second cleavage furrows have reached the lower pole, and the third furrows have just passed the equator (figs. 82 to 84). The second cleavage furrows intersect the first at right angles at the lower pole. For some distance on each side of the pole the second cleavage furrows are for a time markedly wider than the first. The second furrows are further distinguished by the fact that the third furrows run closer to them than to the first. In the latter part of this stage the third furrows sometimes become complete (fig. 84), as a rule joining the first at some distance from the pole.

The biradial pattern of cleavage is thus preserved in the lower hemisphere, and throughout the later cleavage stages affords a trustworthy means of distinguishing first and second cleavage furrows in this region.

The fate of the fourth cleavage furrows that proceed vertically must be studied in later stages. They usually join the second furrow before reaching the lower pole (figs. 92 and 96).

In a given egg the micromeres vary somewhat in size; but a comparison of seventeen carefully drawn camera figures, and the examination of a large number of additional eggs, lead to the conclusion that in this stage there is no regularity in the distribution of large and small cells among the micromeres.

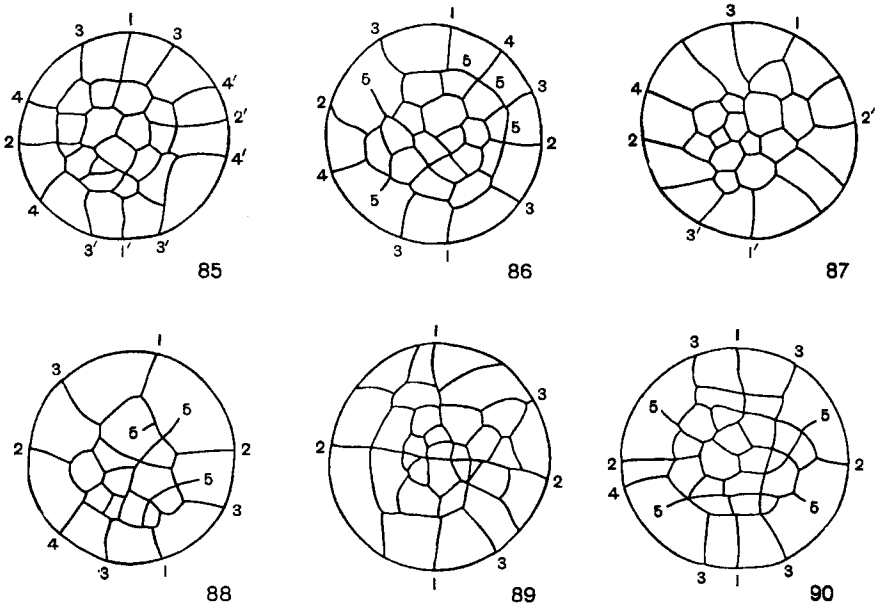
DeBussy's ('04) single figure of the fourth cleavage stage of Cryptobranchus japonicus shows six micromeres surrounded by an approximately circular cleavage furrow, and two recent furrows extending for a short distance vertically.

In Desmognathus, according to Wilder ('04), the fourth cleavage is latitudinal; this conclusion was based on the study of material very limited in amount. Hilton ('09) states that in a large number of eggs he has found only a few which exhibit so regular a type of cleavage as described in Wilder's eight cell and later stages.

In Hynobius (Kunitomo '10), the fourth cleavage furrows are more uniformly latitudinal than in Cryptobranchus allegheniensis. In Necturus (Eycleshymer '04; Eycleshymer and Wilson '10) and Diemyctylus (Jordan '93) a type is no longer recognizable.

In Ceratodus (Semon '00 and '01) the fourth cleavage is latitudinal. Amia (Dean '96; Whitman and Eycleshymer '97) and Lepidosteus (Dean '95; Eycleshymer '99) resemble the type with four micromeres described for Cryptobranchus allegheniensis.

*Stage 5: (figs. 85 to 96; 210 and 211.* This stage is reached about four hours later than Stage 4. It is characterized by the presence of the fifth cleavage furrows, giving a maximum of thirty-two cells, some incompletely divided. More than half of these cells are micromeres.



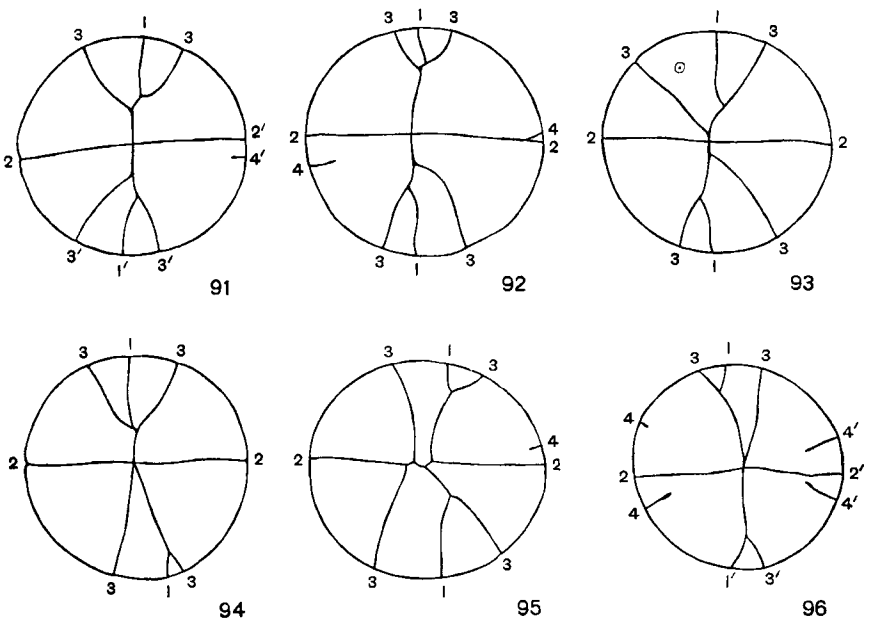

Figs. 85 to 90 Upper hemispheres of eggs of Cryptobranchus allegheniensis in the fifth cleavage stage. All the figures are camera drawings from preserved material. Figure 86 is drawn from the egg photographed for figure 211, and figure 87 from the egg photographed for figure 210. × 4⅔.

The careful study of a large number of eggs emphasizes irregularity in this cleavage and the absence of a well established type. Two sets of fifth cleavage furrows are often recognizable: an inner, within the former region of micromeres, and an outer, just outside of this region. Either set may be, in whole or in part, vertical, latitudinal or oblique (see especially figure 85 for an example of outer, and figure 89 for an example of inner latitudinal cleavage furrows). A study of the most regular cases of cleavage described under Stage 6 shows that in these eggs the fifth cleavage furrows must have come in with greater regularity than in any eggs directly observed in the fifth cleavage stage: these fifth cleavage furrows are almost uniformly vertical, thus preserving the regular alternation in the direction of the furrows.

By the shifting of micromeres the biradial symmetry due to the manner of third cleavage is usually lost in the blastodisc, and unless the egg has been kept under continuous observation it becomes in most cases impossible to trace the first and second cleavage furrows entirely through the region of micromeres.

In preserved material, nuclei are visible from the surface in some of the micromeres of this and the following stages, indicating that these cells are becoming flattened out.

As already noted, in this stage if not in the preceding one, the third cleavage furrows become complete, usually joining the first at some distance from the pole (figs. 91 to 96). This apparent avoidance of the pole by the third cleavage furrow is doubt-




Figs. 91 to 96 Lower hemispheres of eggs of Cryptobranchus allegheniensis in the fifth cleavage stage. All the figures are camera drawings from preserved material. Figure 93 shows a persistent sperm pit (see Part I, Smith '12). × 4⅔.

Fig. 91 Lower hemisphere of the egg whose upper hemisphere is shown in figure 87.

Fig. 96 Lower hemisphere of the egg whose upper hemisphere is shown in figure 85. The fourth cleavage furrows have extended further than is usual in eggs of this stage.

less the mechanical result of the location of the earlier course of the third furrows nearer to the first than to the second; they swerve from the vertical toward the nearest existing cleavage furrow. An analogous pattern may sometimes be observed in the cracking of the corners of a section of cement walk.

I have observed this biradial cleavage pattern in corresponding stages of the lower hemisphere of occasional eggs of Necturus; it is clearly expressed in the cleavage of Desmognathus as figured by Wilder ('04) and Hilton ('04 and '09).

The same tendency to join the nearest existing vertical furrow is shown by those fourth cleavage furrows, as a rule not yet complete, that come in vertically. They usually join the second furrow, at a much greater distance from the lower pole than the intersection of the third with the first.

In the vicinity of the vegetal pole, both first and second cleavage furrows are now only faintly expressed.

In about half the eggs of this stage cell division has proceeded a little more rapidly on one side of the egg than on the other; the cells are smaller in surface view, more numerous, and the cleavage furrows are more uniformly complete (figs. 87, 88 and 210). Thus there is an excentric development of the blastodisc, whereby a condition of bilateral symmetry in the cleavage pattern is produced. This excentric development is a more constant feature in the stages immediately following. The question naturally arises whether this bilateral symmetry in the cleavage pattern has any morphogenetic significance: is it the outward expression of the establishment of the permanent bilateral symmetry and antero-posterior differentiation of the embryo? In other words, does the axis of bilateral symmetry in the cleavage pattern fall in the median plane of the future embryo? This question will be considered in a later paper, in connection with the study of the internal development.

Such an excentric development of the blastodisc has been described for Amblystoma and Necturus by Eycleshymer ('95 and '04), who cites similar observations on other vertebrates by various writers. Eycleshymer speaks of a "second area of accelerated cell division" as distinguished from the primary area

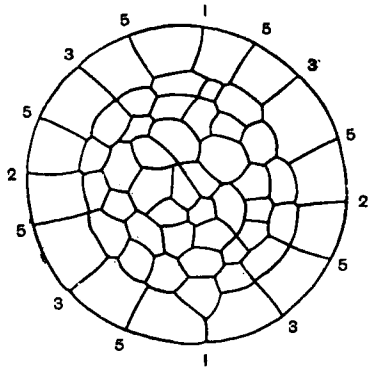
of cell division at the animal pole. In Cryptobranchus what happens seems to be a shifting of the most active area of cell division to an excentric position in the blastodisc; hence I have preferred to speak of it merely as a process of excentric development.

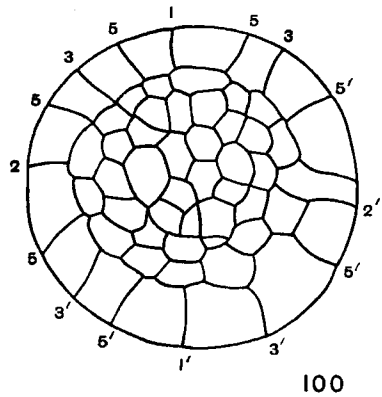
No constant relation exists between the axis of bilateral symmetry due to excentric development and the original direction of the first cleavage furrow as shown by those portions of it that have not undergone shifting. The two may coincide (fig. 88); they may be at right angles to one another; they may be oblique (fig. 87).

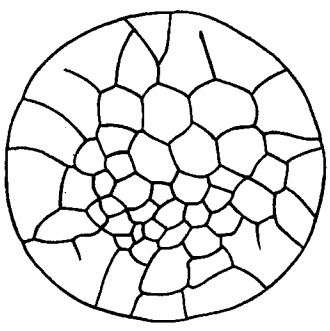
As already indicated, in this stage the cleavage pattern of Necturus bears a strong resemblance to that of Cryptobranchus allegheriensis, but there is this marked difference: the third cleavage furrows of Necturus, when vertical, usually join the first at a greater distance from the vegetal pole, in the region of the equator. In most eggs of Necturus examined in this stage only the first two cleavage furrows extend into the lower hemisphere; these usually meet at right angles at the vegetal pole. Thus the cleavage of Necturus in this stage seems to show an even stronger tendency toward the meroblastic condition. But this is merely a consequence of the tendency for the third cleavage furrows to come in obliquely or latitudinally; a comparison of later stages shows that the meroblastic tendency is in reality a trifle less strongly expressed in Necturus (figs. 107 and 108) than in Cryptobranchus.

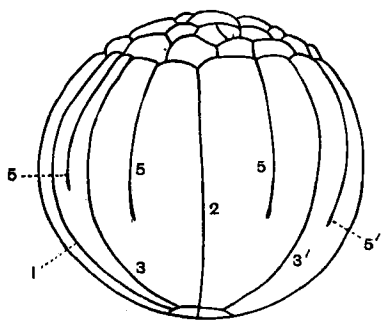
In Amia (Dean '96; Whitman and Eycleshymer '97) the fifth cleavage furrows appear in two sets: an outer set cutting the eight macromeres latitudinally; and an inner set cutting the four micromeres in a horizontol plane, hence not visible from the surface.

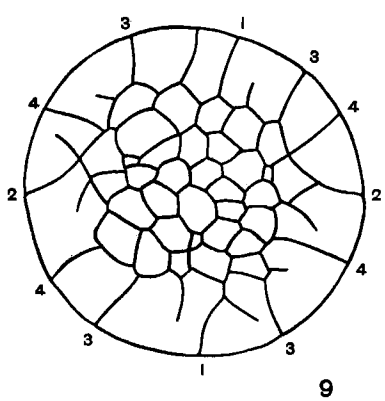
*Stage 6:* (*figs. 97 to 102 and 212 to 214*). This stage is characterized by the presence of the sixth cleavage furrow, giving a maximum of sixty-four cells, some of the macromeres being incompletely divided. Considerably more than half the cells are micromeres; these occupy an area whose diameter extends over only about 90° of the circumference of the egg. Hence the mero-

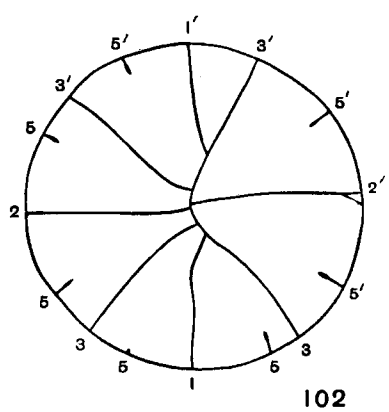



Figs. 97 to 102 Sixth cleavage (Stage 6) of Cryptobranchus allegheniensis. Figures 100 to 102 show upper hemisphere, equatorial view, and lower hemisphere, respectively, of the same egg. All of the figures are camera drawings from preserved material. Figure 98 is drawn from the egg photographed for figure 212. × 7.

blastic tendency is strongly expressed. This stage is reached about four hours later than the beginning of the preceding stage.

A description of a few individual eggs will best indicate the characteristics of this cleavage.

Out of about fifty eggs studied, the one represented in figure 97 shows the greatest regularity of cleavage in the upper hemisphere. This condition must have been reached by a fairly constant alternation of vertical and latitudinal cleavage furrows. This alternation of cleavage furrows, carried out with completeness and geometrical precision would give a total of sixty-four cells, consisting of forty-eight micromeres and sixteen macromeres; the micromeres would be arranged in three concentric rows, each containing sixteen cells. In the egg under consideration, this condition is realized in the outer row of cells, which is quite regular and contains the theoretical number, sixteen. But the total number of micromeres is only thirty-nine, hence a deficiency must exist in the central portion of the blastodisc, or some divisions in this region must have taken place horizontally. Sections show that no horizontal divisions have taken place in this region; on the other hand divisions parallel to the surface have sometimes occurred in the marginal row of micromeres. Therefore cell division is taking place more rapidly in the marginal than in the central region of micromeres—a condition which may be the beginning of that accelerated development of the margin, the later expression of which is almost wholly internal.

A study of other eggs showing a fairly regular alternation of cleavage furrows gives additional evidence for this interpretation (e.g., fig. 100, representing an egg with 39 or 40 micromeres). While eggs with this degree of regularity in the cleavage pattern are the exception rather than the rule, it is felt that evidence derived from them is especially trustworthy; for in such eggs the equilibrium in the rhythmic alternation of the direction of cell division has been best maintained, and the rather uniform lagging-behind of the divisions of the cells in the central area would seem to be the expression of a normal tendency in the life of the embryo. In eggs which show disturbances in this equi-

librium, discordant factors are more likely to be present to obscure the normal expression of the course of development.

The sixth cleavage furrows of the outer set, when latitudinal, divide the macromeres very unequally, cutting off additional micromeres. The number of micromeres, and the extent of the blastodisc, is increased by such latitudinal divisions; the number of macromeres is increased by the sixth cleavage furrows only when these come in vertically.

In a few eggs, as the one shown in figure 99, there is a marked tendency for the sixth cleavage furrows to come in vertically. Here, as noted in an earlier stage, the embryo seems to be oscillating between two possible modes of cleavage; but the tendency to preserve the regular alternation of cleavage furrows is usually the stronger.

The most marked tendency to vary from the regular pattern of cleavage occurs along the line of excentric development of the blastodisc (figs. 98 and 212), as described under Stage 5. The majority of eggs exhibit this tendency in some degree.

We have then, in the cleavage pattern of this stage, two tendencies toward differentiation of the blastodisc: (a) an accelerated cell division in the marginal portion, pointing toward the formation of the germ ring; and (b) an accelerated cell division about a radius of the blastodisc, giving a condition of bilateral symmetry.

DeBussy's figure ('05, fig. 10) representing the blastodisc of an embryo of Cryptobranchus japonicus with forty micromeres strongly suggests excentric development; on one side of the first cleavage furrow only three cleavage furrows reach the equator, on the other side nine. But the author remarks (p. 530) that he has observed no secondary center of accelerated cell division such as has been described by Eycleshymer for Necturus.

A comparison with earlier stages shows that there is an increasing tendency for the micromeres, following a familiar law of developmental mechanics, to lose their original quadrangular or triangular outline and become hexagonal.

In the living egg, the roof of the segmentation cavity is somewhat translucent, and spaces communicating with the cavity

beneath sometimes appear between the cells. Evidently the roof consists of a single layer of flattened cells; this inference is confirmed by the study of sections.

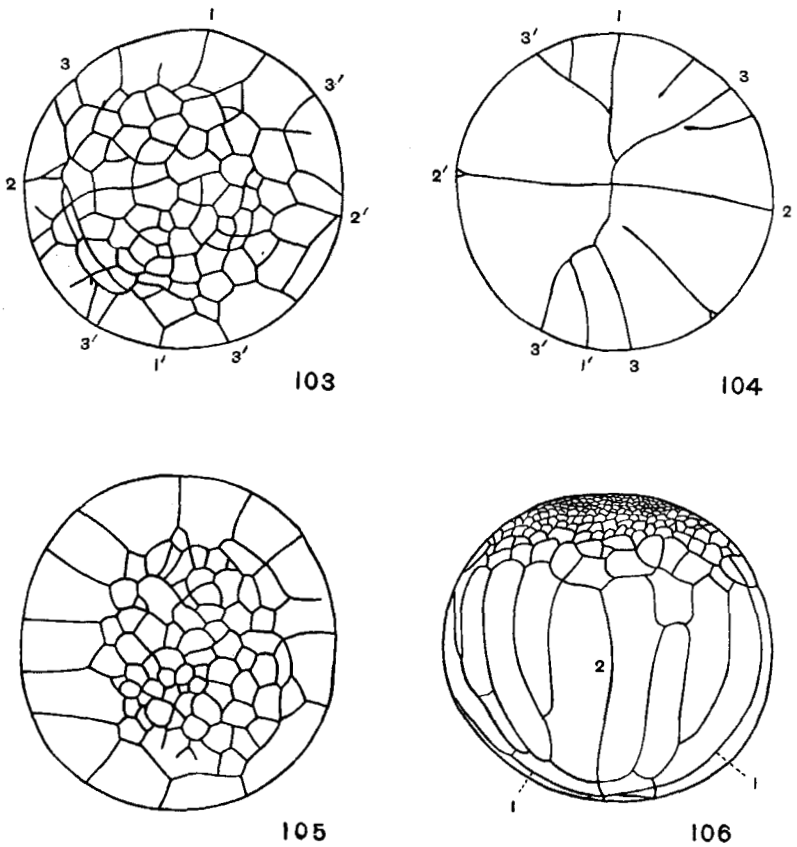
On account of the slow cleavage and relative stability of the macromeres, there is little change in the cleavage pattern of the lower hemisphere. An advance is shown in that the vertical fifth cleavage furrows have invaded the lower hemisphere (fig. 102). Those fourth cleavage furrows that proceed vertically are seldom complete in this stage, but sometimes are found joining an earlier vertical furrow at a considerable distance from the vegetal pole.

In this stage the most regular type of cleavage pattern of Cryptobranchus bears a striking resemblance to the corresponding stage of Amia (Dean '96; Whitman and Eycleshymer '97).

Since Stage 6 of the egg of Cryptobranchus best serves to illustrate the fundamental characteristics of the cleavage pattern, particularly with regard to the relative size of the micromeres and macromeres, at this point a comparison may well be made with the dipnoi and crossopterygii. The general anuran or urodele character of the cleavage of the dipnoan egg is apparent in all existing genera: Ceratodus (Semon '00 and '01); Protopterus (Budgett '01; Kerr '09); and Lepidosiren (Kerr '00, '01 and '09). With respect to inequality in the cleavage, Lepidosiren in particular closely approaches the condition in Cryptobranchus and Necturus. The cleavage of Polypterus (Kerr '07) bears a general resemblance to that of Amblystoma and the frog.

*Stage 7: (figs. 103 to 105; 215 and 216).* This stage is characterized by a doubling of the number of cells found in the preceding stage, and by a slight extension of the region occupied by the micromeres. The stage is reached about four hours later than Stage 6.

Figures 103 and 104 show a fairly representative egg in this stage. The cells in the region of the animal pole are markedly larger than the other micromeres. This condition may be due to one or both of two factors: (a) the flattening of the cells composing the roof of the segmentation cavity; (b) a slower rate of division in these cells, as noted in Stage 6. There is marked




Figs. 103 to 105 Surface views of eggs of Cryptobranchus allegheniensis in Stage 7, showing cleavage furrows. Figures 103 and 104 represent upper and lower hemispheres, respectively, of the same egg. Camera drawings from preserved material. × 7.

Fig. 106 Equatorial view of an egg in Stage 8, showing cleavage pattern. Camera drawing from preserved material. × 7.

activity in cell division in an area excentrically situated, though this is not so apparent in the particular egg under consideration as in some other eggs in the same stage. In the lower hemisphere, the biradial character of the cleavage pattern is preserved and accentuated.

In the egg represented in figure 105 we see the beginning of a process of fundamental importance in the further history of

the embryo—the phenomenon of *immigration of cells from the single-layered roof of the segmentation cavity.* In a surface view, it is evident that some of the cells in the excentrically situated area of most rapid cell division are partially submerged. They are not merely smaller superficially than the other micromeres, but are sunken below the general surface and present the appearance of being crowded inward. Their condition will be further described in a consideration of the internal development; their later history and fate form an important phase of the process of embryo-formation.

In most eggs of this stage, at the margin of the blastodisc oblique furrows (probably fifth cleavage furrows) occasionally cut off cells intermediate in size between micromeres and macromeres. In the lower hemisphere some recent furrows, presumably fifth cleavage furrows, usually extend well toward the vicinity of the vegetal pole. The macromeres are, as a rule, long, narrow and wedge-shaped. On account of the segregation of most of the protoplasm within the region of the blastodisc, all latitudinal divisions of the macromeres are very unequal, cutting off new micromeres instead of increasing the number of macromeres. In the living egg, the roof of the segmentation cavity still appears somewhat translucent, and spaces sometimes occur between these cells; but neither of these conditions is so marked as in the preceding stage.

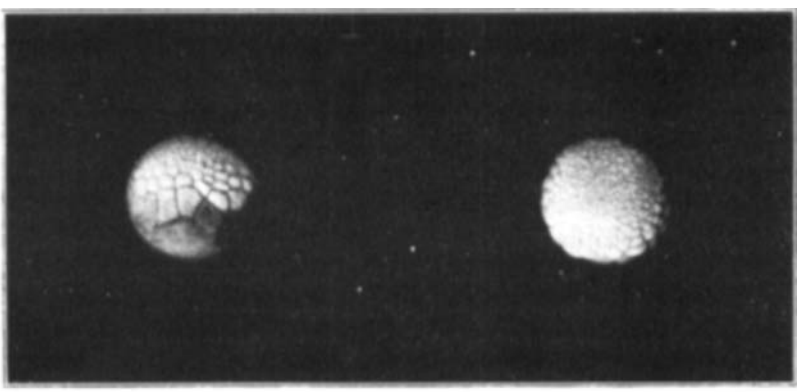
*Stage 8: (figs. 106 and 217).* This stage is reached about twelve hours later than Stage 7; it is best described by reference to the figures. The micromeres have become much more numerous and smaller; there is a slight extension of their area. There is a more gradual transition, or gradation in the size of the cells, between micromeres and macromeres. In the lower hemisphere the fifth cleavage furrows have, as a rule, become complete; they rarely reach the lower pole, but join an earlier furrow at some distance from the vegetal pole. Biradiality of the cleavage pattern still enables one, as a rule, to distinguish first and second cleavage furrows in this hemisphere.

In the upper hem sphere the excentric area of accelerated cell division noted in the preceding stages is usually quite marked.

In preserved material the nuclei of the micromeres are often easily distinguishable in surface views.

In the living egg, the roof of the segmentation cavity has become quite opaque, and the cells are compactly arranged. During the latter part of this stage a translucent condition begins to appear at the animal pole, indicating a thinning-out of the cells in this region, as in Stage 6; but this time the cells form a firm tissue, with no spaces between them.

In Necturus the cleavage furrows in the region of the vegetal pole are fainter than in Cryptobranchus; this condition is reversed



107 108

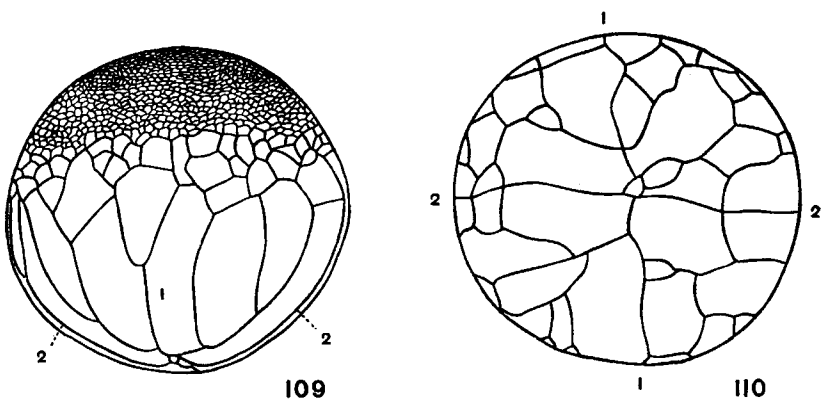
Figs. 107 and 108 Advanced cleavage stage of Necturus maculosus. Two views of a single egg, photographed after preservation. × 4.

in the upper hemisphere, where the micromeres are outlined far more boldly in Necturus (figs. 107 and 108) than in Cryptobranchus (both statements refer to preserved material, fixed by the same method).

*Stage 9:* (*figs. 109, 110 and 218*). This stage is reached about nineteen hours later than the preceding stage. Individual micromeres in the region of the animal pole are barely visible to the naked eye. The zone of transition between micromeres and macromeres has become broader and more marked. An excentrically situated area of accelerated cell division in the micromeres is only occasionally found in surface views of this stage.

In the living egg, the roof of the segmentation cavity appears as a translucent tissue throughout a circular area about 40 degrees in diameter in the region of the animal pole. This indicates a decided thinning-out of the cells of this region.

Biradiality of the cleavage pattern of the lower hemisphere still enables one to distinguish in many embryos, though not in all, the first and second cleavage furrows. Usually two or three cells quite small in surface view occur at the vegetal pole; they are quite characteristic of this and the following stage, but are sometimes found in the preceding stage. At the vegetal

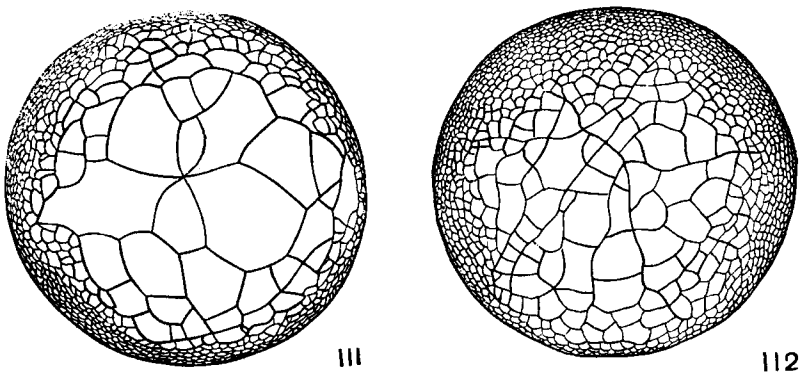



Figs. 109 and 110 Stage 9 of Cryptobranchus allegheniensis. Equatorial view and lower hemisphere of different eggs, showing cleavage pattern. Camera drawings from preserved material. × 7.

pole the cleavage furrows, both in living and preserved material, are sometimes both broad and deep, forming quite noticeable fissures; a similar condition is common in Amblystoma (Eyclesheymer '95). In Cryptobranchus this condition is in marked contrast to the stage immediately preceding, in which the furrows in this region were faint. In Necturus, on account of the variability of the third cleavage furrows, the biradial pattern of the macromeres is not so clearly expressed as in the egg of Cryptobranchus.

*Stage 10: (figs. 111, 112 and 219).* This stage, reached a day or two later than Stage 9, immediately precedes the beginning of gastrulation. The micromeres at the upper pole are invisible

to the naked eye, and barely distinguishable with the magnification used for photographing (× 4). The area occupied by the micromeres extends approximately to the equator, though the broad zone of transition makes it difficult to define. In the vicinity of the vegetal pole, the cleavage furrows have again become faint; in many cases, in preserved material, they are distinguishable as fine lines lighter in color than the general surface, rather than as actual grooves. For the accurate study of these furrows in this and the following stage, a binocular microscope is usually required. When their boundaries are distinct, on account of their large size the macromeres are readily seen with the naked eye.




Figs. 111 and 112 Lower hemispheres of two eggs of Cryptobranchus alleghaniensis in Stage 10, showing cleavage pattern. The embryo shown in figure 112 is slightly older than the one represented in figure 111. Camera drawings from preserved material. In each egg, the lower pole as determined by gravity lies at the center of the figure; the vegetal pole, at the intersection of the first two cleavage furrows, is slightly above this point. The upper part of each figure represents the side on which the blastopore is to appear. × 7.

Usually, the cleavage pattern of the lower hemisphere retains enough of its earlier bilateral symmetry to enable one to distinguish first and second cleavage furrows. The vegetal pole, since it occurs at the intersection of the first two cleavage furrows, may in most cases still be determined quite accurately and conveniently by means of the cleavage pattern. As shown in figures 111 and 112, the vegetal pole is excentrically located in the area occupied by the macromeres; a more rapid multiplication of cells has occurred on one side of this area, so that on this side

the micromeres and transitional cells approach nearer the vegetal pole. A meridian drawn through the vegetal pole and the center of the area occupied by the macromeres defines the axis of excentricity; this axis bears no constant relation to the first cleavage furrow and the axes of biradial symmetry determined by the early cleavage furrows. The biradial symmetry of the cleavage pattern is of course somewhat disguised by the more rapid multiplication of cells at one end of the axis of excentricity.

In this stage occurs a slight tilting of the morphological axis of the egg within a meridional plane determined by the axis of excentricity, so that the vegetal pole no longer coincides with the lower pole as determined by gravity. The vegetal pole is slightly uptilted on the side where the more rapid multiplication of cells occurs, hence the meridian defining the axis of excentricity passes also through the new pole determined by gravity. This new pole at first lies intermediate between the vegetal pole and the center of the area occupied by the macromeres; in later stages, through continued tilting of the egg in the same direction, it comes to lie beyond this center. Throughout the ensuing stages we must distinguish between the morphological axis of the egg as determined by the animal and vegetal poles, and the vertical axis determined by gravity. The method of locating the vertical axis, and exact measurement of the amount of rotation, will be given in the following stage.

If the egg be sectioned along the axis of excentricity the internal structure, to be described later, shows that this axis lies in the sagittal plane of the embryo; the side on which the small cells approach nearer to the vegetal pole is the one on which the blastopore is to appear. Thus the excentric position of the vegetal pole within the area occupied by the macromeres enables one to orient the egg with reference to future body regions.

In perhaps the majority of cases, the transition from large to small cells is more evenly graded on the side where it occurs nearest to the vegetal pole, than on the opposite side where it is characterized by a rather abrupt line of demarcation (figs. 111 and 112). This feature, when present, gives a true bilateral symmetry to the cleavage pattern of the lower hemisphere; the axis of this bilateral symmetry coincides with the axis of excen-

tricity previously defined, hence lies in the sagittal plane of the embryo. The excentric position of the vegetal pole within the area occupied by the macromeres, and the bilateral character of the cleavage in this region, are more marked in many eggs taken immediately after the beginning of gastrulation; these features are usually better expressed than in the eggs shown in figures 111 and 112, which were chosen because the distinctness of the early cleavage furrows enabled them to be drawn with the camera lucida. Schultze ('00, Taf. 11, fig. 12) has described a similar bilaterality in the late cleavage of the lower hemisphere of the frog's egg.

The question of the possible relation of the excentric and bilateral development of the lower hemisphere just described, to the excentric development of the blastodisc noted in previous stages, will be discussed in a later paper.

In the living egg, the roof of the segmentation cavity, though apparently thin, is not quite so translucent as in the preceding stage. It is, however, noticeably more translucent on the side toward the future blastopore, and on this side the transition to the opaque yolk cells is more abrupt.

During the late stages of cleavage, a tendency toward fading out of the cleavage furrows in the vicinity of the vegetal pole has been noted. In some individual cases this process has gone so far that the earlier cleavage furrows are lost to view, even when searched for with the binocular microscope. This tendency may be interpreted as due to a difficulty in sustaining the holoblastic method of cleavage in an egg so heavily laden with yolk. In the corresponding stages of Necturus, this tendency is even more marked. My study of the cleavage pattern of the lower hemisphere of the late segmentation stages of both Cryptobranchus and Necturus has been confined to preserved material, but Professor Dean informs me that he has noticed this merging of lower blastomeres in the late segmentation stages of the living eggs of Necturus. My Necturus material is not so favorable as the egg of Cryptobranchus for the study of the cleavage pattern in this stage, so a detailed comparison will not be attempted.

In the very late cleavage stages of Cryptobranchus japonicus, Ishikawa ('08 and '09) describes a shallow furrow ('Scheidewand-

furche' or 'septal furrow') bounding the posterior margin of the roof of the segmentation cavity, parallel to the future blastopore. Such a furrow does not normally occur in this stage of Cryptobranchus allegheniensis, but a similar furrow makes its appearance shortly after the beginning of gastrulation.

### *B. Summary*

The cleavage is holoblastic, but with great inequality in the size of the micromeres as compared with the macromeres.

Biradial symmetry of the cleavage pattern begins with the third cleavage stage. In the upper hemisphere, as a consequence of the shifting of the micromeres, this biradial symmetry is lost at about the fifth cleavage stage. In the lower hemisphere, because of the slow cleavage and stability of the macromeres, it persists until after the beginning of gastrulation, and in some eggs enables one to distinguish first and second cleavage furrows even after the blastopore has appeared.

An excentrically situated area of unusually small micromeres is apparent in surface views of most eggs in Stages 5 to 8 inclusive; the cleavage pattern of such eggs thus possesses bilateral symmetry.

In the sixth cleavage stage there is more rapid cell division in the marginal region of the micromeres than in the region of the animal pole. The later expression of this tendency is almost wholly internal.

In Stages 7 and 8 some of the superficially smaller micromeres are becoming submerged through a process of immigration.

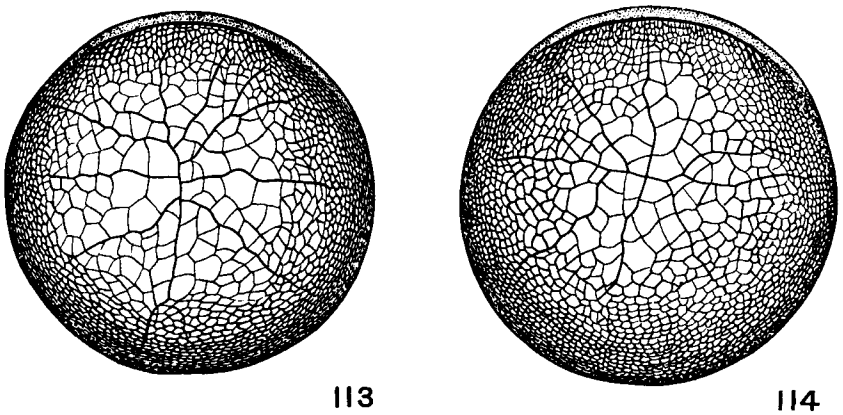
In the later cleavage stages there is a tendency for the cleavage furrows to become less distinct than formerly in the region of the vegetal pole, indicating a difficulty in sustaining the holoblastic character of the cleavage in an egg so heavily laden with yolk; the same tendency is observed in Necturus.

In late segmentation immediately preceding gastrulation the cleavage pattern enables one to predict the side on which the blastopore is to appear; the egg undergoes a slight rotation on a horizontal axis at right angles to the median plane.

## VIII. GASTRULATION AND EARLY FORMATION OF THE EMBRYO

### *A. Description by stages*

*Stage 11: (figs. 113 to 137 and 220 to 222).* This stage extends from the time of the first appearance of the blastopore as a short horizontal groove until its ends meet to form a complete circle. In eggs kept in their natural environment, gastrulation begins about seven days after fertilization and two days after the beginning of Stage 10.




Figs. 113 and 114 Lower hemispheres of two eggs of Cryptobranchus allegheniensis in an early gastrula stage, showing cleavage furrows. The vertical axis as determined by gravity lies at the center of each figure; the vegetal pole, at the intersection of the first two cleavage furrows, is about 7 degrees above the vertical pole. In figure 113 the first cleavage furrow lies approximately in the median plane of the gastrula; in figure 114 it is at right angles to this plane. Camera drawings, finished under the binocular, from preserved material. × 8.

The blastopore is first distinguished as a shallow irregular and broken horizontal groove two or three millimeters in length, lying about 15 degrees below the equator. It occurs at the upper limit of transitional cells between micromeres and macromeres, and its immediate site is distinguished by a rather abrupt demarcation between micromeres and distinctly larger transitional cells. The groove is started, not by a lining-up of cells and the union of cleavage furrows, as described by Eycleshymer ('95) for Amblystoma, but by the sinking-in of groups of entire cells

at intervals along a narrow zone several cells in width; hence from its very beginning the process is not a splitting-apart of cells, but invagination. The groove soon becomes continuous and deepens by the inturning of cells along both margins.

After the groove has reached a length of three millimeters or more, the process of invagination becomes accompanied by one of overgrowth or epiboly: the dorsal lip grows slowly down over

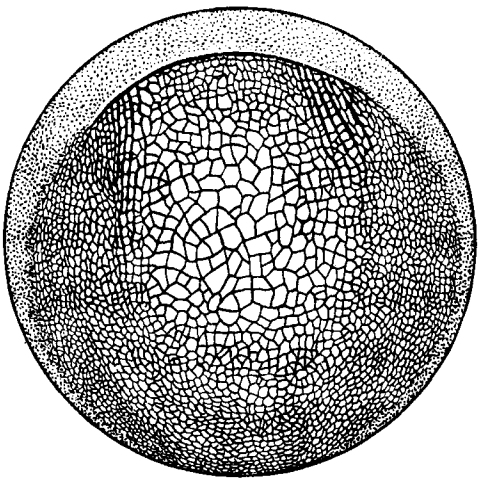


Fig. 115 Lower hemisphere of a gastrula of Cryptobranchus allegheniensis, in a slightly later stage than the preceding, showing the lining-up of the cells within the horns of the blastopore. Freehand drawing from a photograph of preserved material. × 10.

the cells transitional between micromeres and macromeres (figs. 113 to 115). As shown in figure 115, the transitional cells just within the horns of the blastopore are elongated as if compressed; here the cells line up and lengthen out at right angles to a line connecting the horns of the blastopore.

After the blastoporic groove has attained the form of a semicircle (fig. 133), a zone of rather abrupt demarcation between micromeres and transitional cells completes the circle begun by the crescentic blastopore; this zone marks the site of the ventral lip of the blastopore. A little later, the blastoporic groove extends rapidly along this line of demarcation, becoming an almost

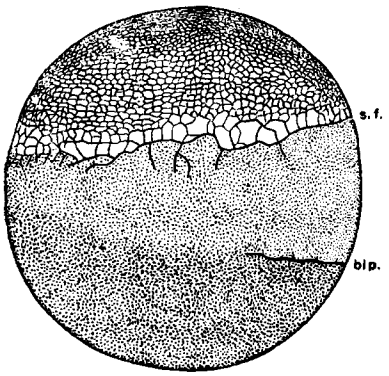
perfect circle, and enclosing a broad horseshoe-shaped band of transitional cells, within which lie the macromeres (fig. 138).

It has already been noted that a slight rotation of the egg on a horizontal axis has taken place in Stage 10, so that it is now necessary to distinguish between the morphological axis of the egg and the vertical axis determined by gravity, since the two no longer coincide. In the study of gastrulation this rotation must be taken into account, and some means must be found for measuring it. In studying the morphological features of the egg (position of blastopore, etc.) in their relation to the vertical axis, two general methods have been used: (a) the living egg, placed in a small vial of water, has been studied in side view and measurements made against a protractor used as a background; and (b) for accurately locating the vertical axis I have devised the following apparatus: a glass disc such as is used for an ocular micrometer was marked in the center with a small dot; a circle with a radius of 4 mm. was then drawn about this dot as a center. When this disc is placed in the eyepiece of a low-power microscope used in studying the eggs, the circle is just large enough to enclose the image of an egg. When the egg, immersed in water in a watch glass, is accurately placed so that its image is enclosed by the circle, the dot lies over the upper pole of the vertical axis; this point is then marked by puncturing with a hot needle. The operation was first tried on living eggs, which were then fixed for further study; but since with the living egg even a small puncture in this region usually causes the embryo to collapse during the subsequent process of fixation, in general this method is less satisfactory with living than with preserved material. On account of the usually perfect preservation of the form of the egg by the fixing fluid employed, the results obtained by marking preserved material seem fairly trustworthy, especially when spherical eggs are selected and a large number used. The position of the upper vertical pole, thus marked, gives a reference point for correlating the morphological features of the egg with the vertical axis; the measurements were made by means of camera drawings.

In making these measurements, it is especially necessary to guard against using eggs with an unusually large yolk plug, since this is one of the commonest abnormalities. Moreover, even in perfectly normal eggs there is considerable variation in the position of the blastopore, so that a large number of eggs must be studied and averages taken. The results obtained by the two methods agree closely.

Since the blastopore, at the time of its first appearance is only about 15 degrees below the horizontal equator and approximately parallel to it, the blastopore at first forms an arc of an imaginary circle whose diameter, measured along a meridian of the egg, is about 150 degrees. At the time when the blastopore has reached the form of a semicircle, this diameter measures about 125 degrees; when the blastopore has become a complete circle the average diameter, in normal embryos, is only 94 degrees. Therefore the crescentic blastopore forms an arc of a circle of steadily diminishing diameter; the lips of the blastopore, and the entire germ ring (to be described in a later paper), contract as they progress slowly downward over the egg.

In preserved material the cleavage pattern of the macromeres is still fairly well defined; by means of careful study with a binocular microscope it is usually possible to distinguish first and second cleavage furrows (figs. 113 and 114). This enables a direct comparison to be made between the direction of the first cleavage furrow and the median plane of the gastrula; this point will be discussed in a later paper. The identification of early cleavage furrows in this region is furthermore of importance in enabling one to determine the position of the vegetal pole, since this is located at the intersection of the first two cleavage furrows. Measurements show that at the time when the blastopore is first clearly established, the vegetal pole lies, on the average, 68 degrees below it, and 7 degrees above the lower pole of the vertical axis. At the time when the blastopore becomes a semicircle, the vegetal pole lies only 32 degrees below its dorsal lip; when the blastopore first becomes a complete circle the vegetal pole lies only 26 degrees below the dorsal margin of the yolk plug. During this time continued rotation of the egg has brought its

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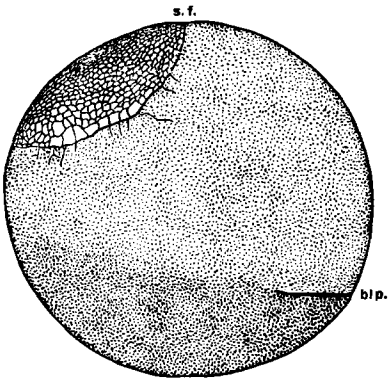


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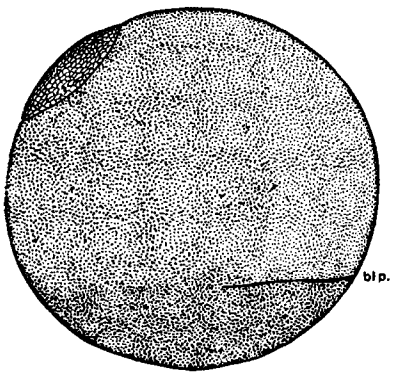


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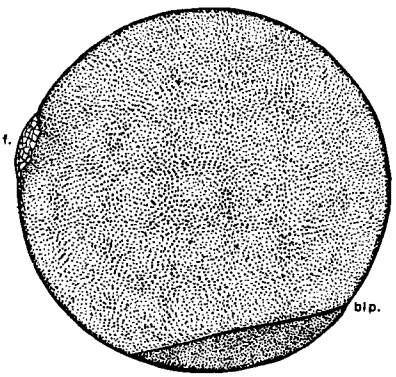


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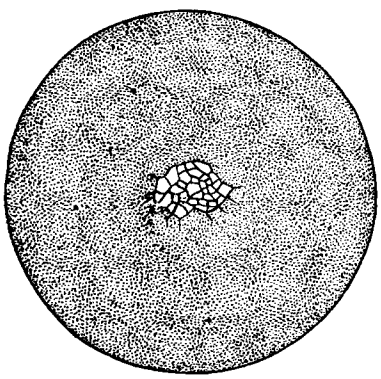

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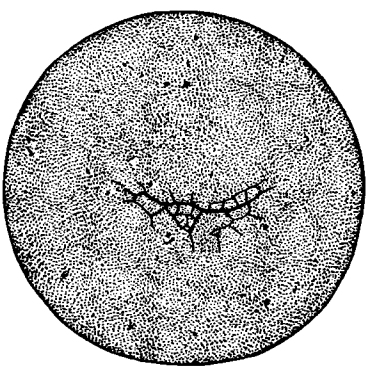

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morphological axis to an angle of 44 degrees from the vertical; the ventral lip of the blastopore now lies about 24 degrees beyond the lower pole of the vertical axis. These changes are set forth diagrammatically in figures 134 to 137.

Two quantitative results of considerable importance are brought to light through the study of these data: (a) the dorsal lip of the blastopore has grown downward over the yolk cells for a distance of about 42 degrees; (b) the egg has rotated in the opposite direction about 37 degrees from its position at the beginning of gastrulation, making a total rotation of 44 degrees. At first, overgrowth is more rapid than rotation; at the time when the blastopore has reached the form of a semicircle its dorsal lip is 43 degrees below the horizontal equator. Later, rotation is more rapid than overgrowth, and at the time when the blastopore has become a complete circle its dorsal lip has been carried back to a position 20 degrees below the horizontal equator, only 5 degrees lower than its original position in space.

The changes in the upper hemisphere visible from the surface during the establishment of the blastopore are remarkable, since they afford clues to many important processes within. In the living egg especially, because of the translucent character of the upper hemisphere, one is able to get total views of many phases of gastrulation, such as could not be obtained from serial sections except by means of reconstructions. Except where otherwise noted, the following description is based on the study of the living egg.

At the very beginning of the process of gastrulation, the nearly transparent roof of the segmentation cavity is of quite uniform

Figs. 116 to 121 Stage 11 (gastrula) of Cryptobranchus allegheniensis. Camera drawings from preserved material. In all the figures, the upper vertical pole as determined by gravity lies toward the top of the page; *blp.*, dorsal lip of the blastopore; *f.*, fenestra (roof of the blastocoele differentiated into a window-like structure); *s.f.*, septal furrow. × 7½.

Fig. 116 Lateral view of an early gastrula stage. The sharp differentiation of the fenestra is rather precocious in this egg.

Figs. 117 to 119 Lateral views of a characteristic series of later embryos.

Figs. 120 and 121 Antero-ventral views showing stages in the disappearance of the fenestra. Figure 120 is from the egg drawn for figure 119.

extent about the animal pole as a center, covering an area about 140 degrees in diameter. As gastrulation advances this clear area becomes encroached upon at its posterior margin (figs. 122 and 123) by the extension of the opaque material. Meanwhile the boundary of the roof of the blastocoele becomes more sharply defined; before the upgrowth of the postero-dorsal opaque region has reached the animal pole the margin of the blastocoele roof is usually bounded by a sharply defined furrow, the 'septal furrow' of Ishikawa (see below)—a characteristic and almost unique feature of the gastrulation of Cryptobranchus. The precise stage at which this furrow appears varies considerably in different eggs; figure 116 shows a case of unusually early appearance, figures 117, 125 and 126 a stage in which it is usually well established. Moreover, the distinctness of this groove varies greatly, particularly in eggs of different spawnings; in some lots of eggs the groove is established early and is very sharply marked, while in occasional lots of eggs it is almost absent.

The septal furrow appears first at the posterior margin of the roof of the segmentation cavity, then extends gradually around to its anterior margin; in its appearance and manner of extension

Figs. 122 to 133 Stage 11 (gastrula) of Cryptobranchus allegheniensis. Free-hand drawings of the living eggs, viewed by both transmitted and reflected light; the proportions of the various parts are checked by comparison with camera drawings of preserved material. The drawings are oriented with respect to the vertical axis determined by gravity. The roof of the segmentation cavity is nearly transparent; the roof of the gastrocoele is quite translucent, or slightly opaque in the regions containing mesoderm; heavily yolk-laden regions are decidedly opaque; *bc.*, roof of blastocoele; *blp.*, dorsal lip of the blastopore; *f.*, fenestra (roof of the blastocoele differentiated into a roof-like structure); *gc.*, gastrocoele; *m.*, region containing mesoderm. × 5.

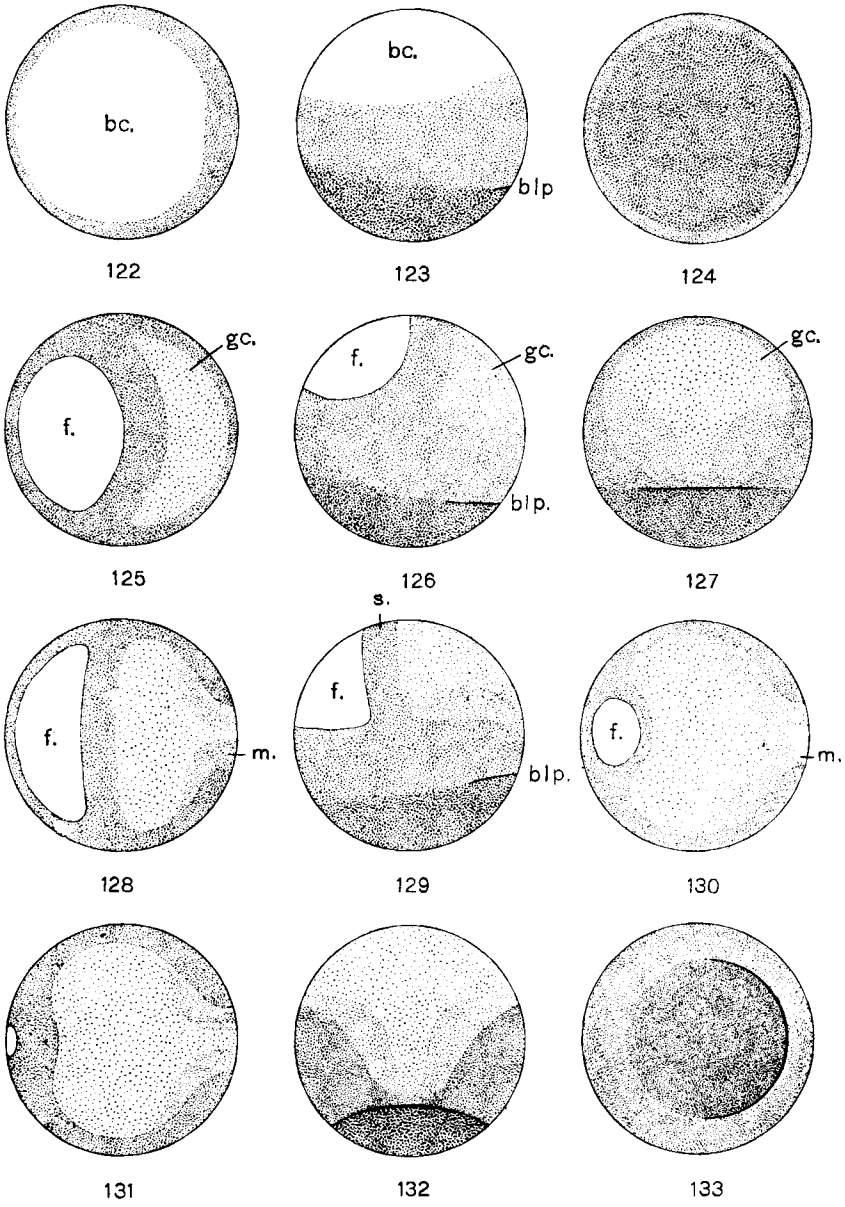
Figs, 122 to 124 Upper hemisphere, lateral view and lower hemisphere of an egg in the beginning gastrula stage.

Figs. 125 and 126 Upper hemisphere and lateral view of an egg a little later than the preceding.

Figs. 127 to 129 Postero-dorsal view, upper hemisphere and lateral view of an egg slightly later than the preceding.

Fig. 130 Upper hemisphere of a slightly later egg.

Figs. 130 to 133 Upper hemisphere, postero-dorsal view and lower hemisphere of an egg near the close of Stage 11 (shortly before the appearance of the neural groove).


bc.
122
bc.
blp
123
124
gc.
f.
125
f.
gc.
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126
gc.
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s.
f.
m.
128
f.
blp.
129
f.
m.
130
131
132
133

it somewhat resembles a blastopore (fig. 221). Later, the groove becomes faint at its posterior margin, very pronounced at its antero-ventral margin (fig. 222). The area enclosed by the groove diminishes in size with its forward movement; it also becomes almost transparent. Since throughout the remainder of its history this area strikingly resembles a window, I shall refer to it as the *fenestra*.

In material fixed in a modification of the bichromate-acetic-formalin mixture (see Smith '12, Section III, Solution B) containing twice the usual amount of potassium bichromate, the fenestra is cut up into small polygonal areas separated by furrows that greatly resemble cleavage furrows (figs. 116 to 120 and 222; cf. Ishikawa, '08 and '09). These polygonal areas do not represent single cells; each comprises a group of several cells. The phenomenon is not entirely an artifact, since it often appears, though faintly, in the living egg. By this method of fixation the septal furrow is likewise accentuated.

Before describing the further history of the fenestra it is desirable to direct attention to some other changes in the upper hemisphere as observed in the living egg.

About the time that the fenestra becomes limited to the anterior half of the upper hemisphere by the upgrowth of the posterior margin of the opaque region, a translucent area, the roof of the gastrocoele, appears in this region of upgrowth (figs. 125 and 126). This translucent area is at first crescent-shaped; it is separated from the more transparent fenestra by an opaque band which is the outward expression of the septum separating the gastrocoele from the blastocoele.

As soon as the septum has advanced into the anterior half of the upper hemisphere, the translucency of the roof of the gastrocoele extends backward almost to the blastopore—evidently by the deepening of the gastrocoele in this region, admitting light. Meanwhile each postero-lateral margin of this region becomes bordered with a faint band of a slightly more opaque character—an effect due largely to the early mesoderm (figs. 127 to 129), though the entoderm is also concerned in producing it.

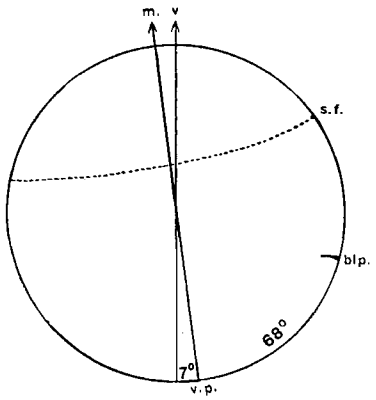
Later changes are concerned with the forward, or rather ventrad, progress of the septum and the increase in the extent of the translucent roof of the gastrocoele, with a correlated ventrad movement of the fenestra and a diminution of its area; there is a slight increase in the extent and opacity of the mesoderm (figs. 118 to 120 and 130 to 132). The fenestra finally closes just below the horizontal equator on the ventral side of the egg (fig. 121). The changes in the position and extent of the fenestra are shown diagrammatically in figures 134 to 137.

The foregoing detailed account of the progress of the septum as viewed from the exterior in the living egg of Cryptobranchus clears up whatever doubt may exist as to the significance of the 'shadowy area' described in the gastrula of Spelerpes by Goodale ('11) and confirms his suggestion as to the nature of this area.

Ishikawa ('08 and '09) describes in the early gastrula of Cryptobranchus japonicus a furrow bounding the roof of the blastocoele at its posterior margin, which he calls the 'Scheidewandfurche' or 'septal furrow.' As compared with the furrow of similar nature described above for C. allegheniensis, it is earlier in making its appearance, since it antedates the blastopore. The area later enclosed by this furrow has been named by Ishikawa the 'Keimhohlensegment' or 'blastocoele-segment'; judging from his figures its later history is much the same as that of the corresponding structure, which I have preferred to call the 'fenestra,' in Cryptobranchus allegheniensis.

The only mention of similar structures which I can find in the literature on other forms is a description by Hatta ('07) of a groove which he calls the 'boundary groove' in the gastrula of Petromyzon. As compared with the septal furrow of Cryptobranchus this groove is greatly exaggerated in Petromyzon, constricting the egg so that in some cases it assumes an hour-glass form.

As suggested by Hatta, the boundary groove or septal furrow is passive in origin, and a product of gastrulation. Similar conditions have produced it in two such widely separated forms as Cryptobranchus and Petromyzon; in each case the egg contains considerable yolk, and the roof of the blastocoele is unusually

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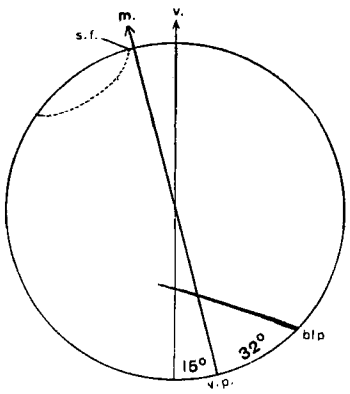


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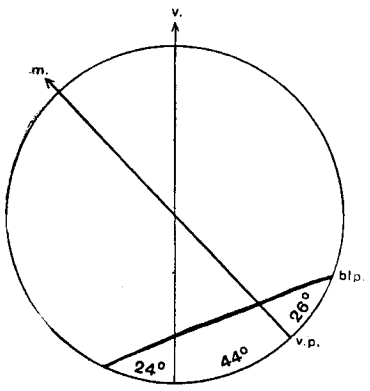


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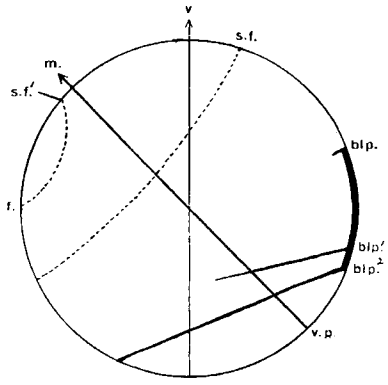


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Fig. 134 Diagram of an egg of Cryptobranchus allegheniensis in the beginning gastrula stage viewed from the lateral aspect, showing average amount of rotation, and the positions of the beginning blastopore and the septal furrow; *blp.*, blastopore; *m*, morphological axis; *s.f.*, septal furrow; *v*, vertical axis determined by gravity; *v.p.*, vegetal pole.

Fig. 135 Similar diagram of a gastrula at the time when the blastopore reaches the form of a semicircle. Lettering as before.

Fig. 136 Similar diagram of a gastrula at the time when the blastopore first becomes a complete circle. Lettering as before.

Fig. 137 Combination of the preceding diagrams. The egg is shown in the position assumed at the close of the period considered. The black band indicates the amount of overgrowth of the dorsal lip of the blastopore (42 degrees); *blp.*, *blp'*, and *blp*², mark the successive positions of the dorsal lip of the blastopore; *s.f.* and *s.f.'*, successive positions of the septal furrow; *f*, position of the vanishing fenestra. Other lettering as in the preceding figures.

thin. As stated by Ishikawa, the polygonal figures formed on the surface of the blastocoele-segment (fenestra) are perhaps due to the pressure which produces the gradual diminution of its area; but the cells of the fenestra are not compacted together to any considerable extent, since the gastrocoele roof and wall merely grow under them.

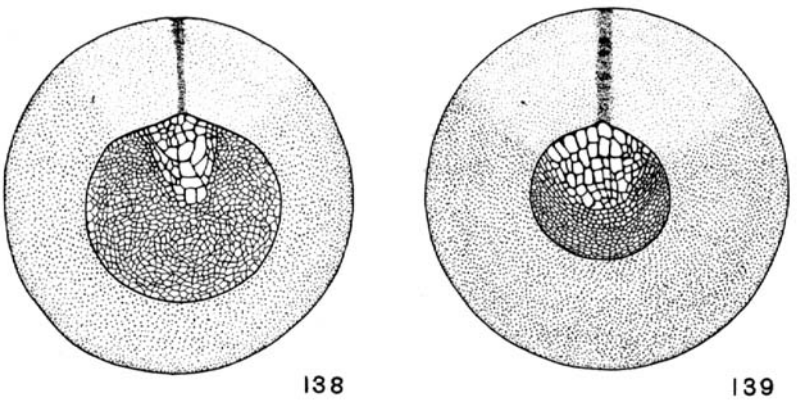
In view of the later history, it is evident even from surface views that in the stage shown in figures 116 and 123 the formative material for the embryo is mainly concentrated in the equatorial region as a broad band or zone of cells, wider in its posterior portion. As will be shown in the description of the internal structure, this equatorial zone as distinguished in surface views is only roughly comparable to the germ ring of fishes.

My material is lacking for the study of the early gastrula stages of Necturus; late gastrula stages differ from Cryptobranchus chiefly in that the blastopore earlier becomes a complete circle. In Spelerpes, according to Goodale ('11), no ventral lip is formed to the blastopore. As compared with urodele and anuran eggs in general, the blastopore of Cryptobranchus is late in closing; in its mode of gastrulation the egg of Cryptobranchus approaches more nearly the type observed in meroblastic eggs.

*Stage 12: (figs. 138 to 150 and 223 to 225).* This stage is characterized by the presence of the neural groove and is terminated by the appearance of the neural folds. The neural groove appears about three days after the beginning of gastrulation.

At the time of the earliest indications of the neural groove, the blastopore has just become a complete circle. At the close of the preceding stage it had a diameter of about 94 degrees; it now rapidly becomes smaller, so that before the appearance of the neural folds its diameter averages about 26 degrees (figs. 138 to 145).

During the early part of this stage the yolk plug is characterized by a broad crescent-shaped or horseshoe-shaped area of smaller cells lying ventrad and laterad to the macromeres (figs. 138 and 139). Along the lateral line of transition between the macromeres and these smaller cells, the cells appear compressed and exhibit a tendency to line up and merge their cleavage furrows (figs.




Figs. 138 and 139 Posterior views of embryos of Cryptobranchus allegheniensis in Stage 12, showing the condition of the blastopore and the cleavage furrows of the yolk plug. Camera drawings from preserved material. The embryos are not accurately oriented with respect to the vertical axis determined by gravity. × 7.

Fig. 138 Showing condition shortly after the appearance of the neural groove.

Fig. 139 A little later than the preceding.

138 to 142; cf. figs. 113 to 115). Toward the close of this stage, the smaller cells become completely overgrown by the ventral and lateral lips of the blastopore, leaving only the larger ones exposed (fig. 144); evidently overgrowth is now proceeding more rapidly at the ventral than at the dorsal lip of the blastopore. At the close of the stage the greatly reduced yolk plug lies entirely on the postero-dorsal side of the lower pole of the vertical axis.

During the earlier part of the stage under consideration the closing fenestra often persists as a pit or small tract of distinct

Figs. 140 to 145 Dorsal views of embryos of Cryptobranchus allegheniensis in Stage 12, showing a series of stages in the development of the neural groove. Camera drawings from preserved material. The embryos are not oriented with respect to the vertical axis determined by gravity. × 7.

Fig. 140 Showing earliest appearance of the neural groove.

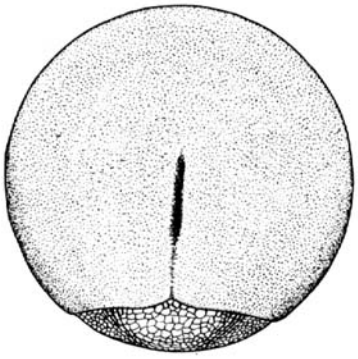
Fig. 141 Slightly later than the preceding.

Fig. 142 Slightly later than the preceding, showing segmented neural groove.

Fig. 143 Slightly later, segmented neural groove. See also figure 225 from the same embryo.

Fig. 144 Late neural groove.

Fig. 145 Showing the condition of the neural groove at the time of the first faint indications of the neural folds.



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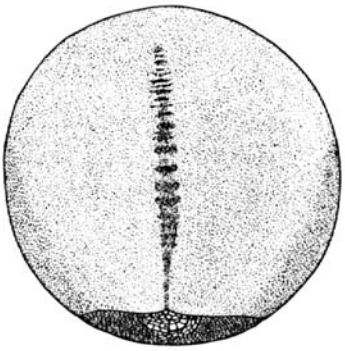

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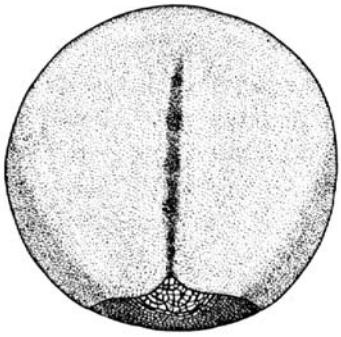

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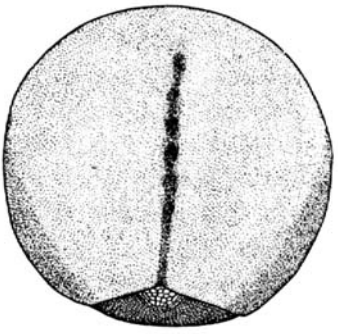

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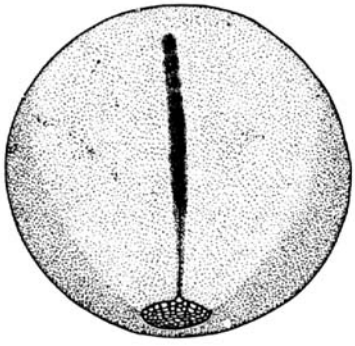

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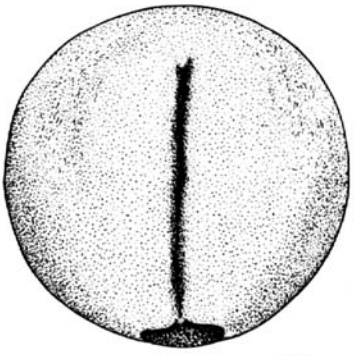

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furrows at the equator on the antero-ventral side of the egg. It usually disappears by the time the neural groove is well established.

In preserved material, the roof of the gastrocoele is considerably paler than the remaining surface of the egg; during the latter part of this stage the neural plate is usually differentiated as a spatulate area extending from the dorsal lip of the blastopore to a little distance in front of the upper pole of the vertical axis, and distinguishable through the greater whiteness of its surface (see especially figs. 223 and 224).

The dorsal lip of the blastopore is not a perfect arc of a circle, but is somewhat incurved on each side of a forward-extending notch in the median line (figs. 138 to 145).

At the time of its first appearance, the neural groove occurs as a distinct furrow extending *from the notch in the dorsal lip of the blastopore* forward in the median line for a distance of about 60 degrees; the anterior half is much broader and deeper than the posterior half (fig. 140). In a slightly later stage, the neural groove has extended to a total length of about 95 degrees but is nowhere so deep as in the anterior half during the preceding stage (fig. 141). It is now a rather shallow groove, narrow in its posterior portion, wider and more broken by occasional deeper depressions or fissures in its middle and anterior parts. These early transverse furrows do not occur at very regular intervals, and are probably only incidental to the process of infolding of the tissues.

A little later, the neural groove becomes decidedly deeper in its middle portion (fig. 142). The change is not uniform throughout this region, but instead there is a series of three or four large pits or depressions at fairly regular intervals, giving a segmented appearance to the groove. Sometimes this segmented condition is very marked; it has been repeatedly observed in living material. Gradually the segmented region, though less sharply marked becomes more extensive than before (fig. 143); it is best seen in living material viewed by transmitted light, when the neural groove appears made up of a regular succession of alternate light and dark areas. Shortly before the appearance of the neural

folds, the neural groove becomes conspicuous in its anterior as well as its middle portion by the broadening and deepening of the former region; at the same time the posterior end becomes fainter (fig. 144).

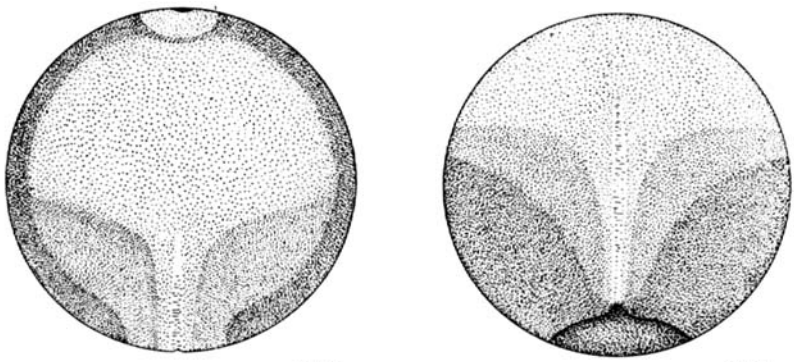
At the time of the first faint indications of the neural folds, the neural groove is both broad and deep throughout its entire length but especially in its anterior portion (fig. 145). During these later changes in the breadth and depth of the neural groove there has been very little increase in length; at the close of the period considered it has a length of about 105 degrees and extends from the dorsal lip of the blastopore nearly to the upper pole of the vertical axis.

According to Griggs ('10), in Amblystoma the first groove to appear in the median line of the neural plate is not the neural groove, properly speaking; this appears later on the same site. There first appears a 'posterior germinal depression' which does not reach the blastopore; a little later an 'anterior germinal depression' is formed, which is discontinuous with the earlier groove. In a later stage both give place to the neural groove which is a different structure on the same site.

In Cryptobranchus the earliest groove to appear in the median line of the neural plate extends forward without break from the dorsal lip of the blastopore. The marked depression shown in figure 140 probably corresponds to the 'posterior germinal depression' in Amblystoma; the later depression in the anterior portion of the neural groove perhaps corresponds to the 'anterior germinal depression,' but it is at no time sharply separated from the remainder of the groove. The later history of the groove will be given in the following stages and should be consulted in this connection; but it may here be stated that after a careful study of both surface views and serial sections I have come to the conclusion that the differences in the grooves appearing early and late in the median line of the neural plate of Cryptobranchus are differences in degree, not in kind, hence I have used the term 'neural groove' throughout.

In living material the embryo may be viewed by transmitted light. During the early part of this stage (figs. 146 and 147)

the broad lateral bands lying in the posterior part of the egg at some distance from the median line are more marked than in the preceding stage. The study of sections shows that they are due to the combined optical effect of the mesoderm and an unusually thick region of the entoderm. The neural groove is particularly translucent. The greatly reduced blastocoele persists in the region of the equator on the antero-ventral side of the egg; the center of its external wall is marked by a pit, the vestige of the fenestra. During the latter part of Stage 12 (fig. 148) the




Figs. 146 and 147 A living egg of Cryptobranchus allegheniensis in an early neural groove stage, viewed so far as possible by transmitted light. Figure 146 shows the upper hemisphere, figure 147 a postero-dorsal view. × 7.

lateral bands are obscured by the thickening of the neural plate; in the central portion of the neural groove there usually appear a series of translucent pits arranged at regular intervals. The pit marking the site of the closing fenestra has disappeared, but there usually remains a translucent area indicating a vestige of the blastocoele; this area is often imperfectly separated from the translucent roof of the gastrocoele.

In this stage it is usually impossible to identify cleavage furrows in the yolk plug, but the stability of the larger cells and the fact that they remain longest exposed afford a means of locating approximately the vegetal pole. We have seen that in the preceding stage the vegetal pole was situated a little above the center of the area of largest macromeres; at the close of Stage 12 these

largest macromeres are the only ones exposed, and we may feel quite sure that the vegetal pole lies in their midst and probably very near the center of the greatly diminished yolk plug. The morphological axis of the egg is thus approximately determined by a line passing from the center of the yolk plug through the center of the egg, and at the close of Stage 12 this axis makes an angle of 52 degrees with the vertical—showing that rotation has proceeded 8 degrees further than in the preceding stage (fig. 149).

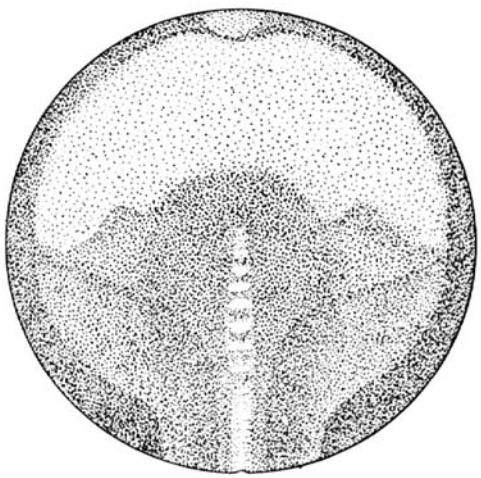
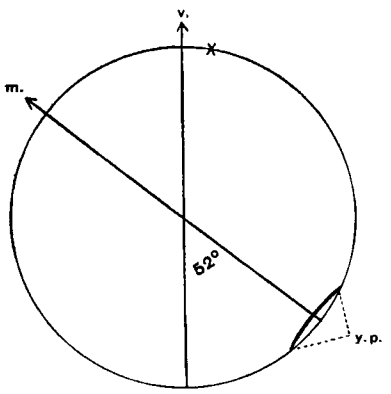


Fig. 148 Upper hemisphere of a living egg of Cryptobranchus allegheniensis viewed so far as possible by transmitted light, shortly before the appearance of the neural folds. × 9.

It will be recalled that the dorsal lip of the blastopore first appears, on the average, 68 degrees above the vegetal pole (fig. 134), and that the ventral lip is first formed about 68 degrees from the vegetal pole on the opposite side (fig. 136). Hence the dorsal and ventral lips are formed respectively at approximately equal distances from the vegetal pole, though the ventral lip is formed much later than the dorsal. We may now compute the amounts of overgrowth of the dorsal and the ventral lips respectively: at the close of Stage 12 the dorsal lip has overgrown the yolk for an average distance of 55 degrees, and the ventral lip has advanced toward it through an arc of about 55 degrees;

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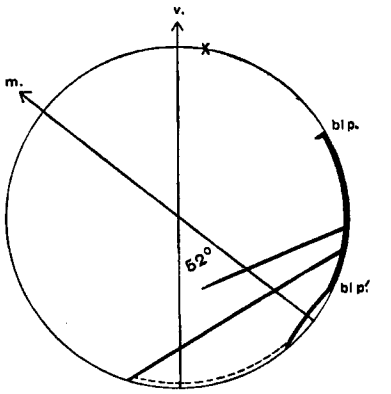


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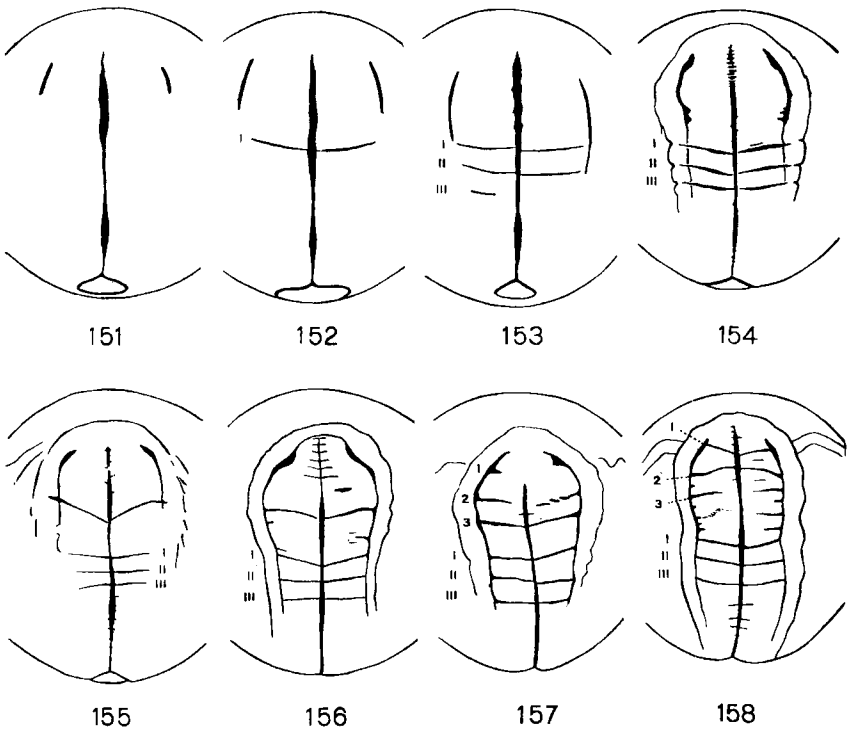
Fig. 149 Diagram of an egg of Cryptobranchus allegheniensis at the close of Stage 12, showing the amount of rotation and the position of the yolk plug; *m*, morphological axis; *v*, vertical axis determined by gravity; *y.p.*, yolk plug. The cross indicates the position of the anterior end of the neural groove.

Fig. 150 Combination of figure 144 with some features of figure 137, showing successive positions of the blastopore; *blp* and *blp'* respectively indicate early and late positions of the dorsal lip of the blastopore. The black band at the right of the figure indicates the amount of overgrowth (55 degrees) of the dorsal lip of the blastopore; the dotted line indicates the amount of overgrowth (55 degrees) of the ventral lip.

that is to say, the distances are approximately equal (fig. 150). By far the greater amount of overgrowth of the dorsal lip occurred during the preceding stage, hence it is clear that during the present stage overgrowth is taking place much more rapidly at the ventral than at the dorsal lip of the blastopore.

*Stage 13: (figs. 151 to 164 and 226 to 228).* The most conspicuous changes during this stage are those concerned with the formation of the neural folds and the segmentation of the neural plate. The neural folds begin to form about one and one-half days after the appearance of the neural groove. During the progress of this stage the neural groove becomes most conspicuous in an anterior and a posterior portion, separated by a middle region in which it is comparatively faint (see especially figs. 151 to 155).

The early stages in the formation of the neural folds are shown in figures 151 to 158 and need no further description, save to men-




Figs. 151 to 158 Stage 13. A series of embryos of Cryptobranchus alleghe-niensis showing early stages in the formation of the neural folds and the segmentation of the neural plate. Camera drawings, finished under the binocular, from preserved material. I, II and III indicate the earliest transverse grooves; 1, 2, 3, grooves appearing a little later, numbered consecutively and not in the order of appearance. × 6.

tion that the surface just outside of the neural folds becomes very much roughened and traversed by fissures parallel to the folds, indicating stresses and the rapid shifting of material. During the later part of this stage a pair of less conspicuous transverse folds appear lateral to the anterior end of the neural plate (fig. 158); the significance of these folds has not yet been determined with certainty (but see Stage 15).

The first transverse groove to cross the neural plate is shown in figure 152. A little later, two transverse grooves appear in rapid succession posterior to it. These first three transverse

grooves are equidistant, and so distinct that they may readily be seen with the naked eye; since they regularly appear in the same order and position in different embryos, and persist throughout the further history of the open neural plate, they serve as trustworthy landmarks during the following stages. In the figures they are numbered with Roman numerals. By following their history through later stages they have been traced to the region of the medulla oblongata of the adult brain; consequently, at least all that portion of the neural plate in front of Groove III belongs to the cephalic plate.

The early segmentation of the cephalic plate in front of Groove I will now be considered. There first appears a transverse groove dividing this region into two portions of which the posterior is slightly the smaller (figs. 155 and 156); the anterior of these areas is then crossed by two more grooves (figs. 157 and 158), while the posterior area is for the present doubtfully segmented. The smaller transverse grooves occurring in various parts of the cephalic plate are irregular in position and probably are of no segmental value; most of them disappear in later stages. Those grooves in front of Groove I which are regarded as of metameric value are numbered with Arabic numerals, consecutively and without regard to the order of appearance.

The question naturally arises whether these early transverse divisions of the cephalic plate are neural in origin or secondarily produced by the segmentation of the underlying mesoderm. This question has not yet been thoroughly investigated by the study of sections, but the results of a preliminary examination favor the idea that in front of Groove I at least, they are primarily neural structures; the mesoderm, particularly in front of Groove I, is at this time quite thin as compared with the neural plate, and hardly capable of producing the modifications of the latter layer.

Since Grooves I, II, III, etc. (see also Stage 14) are produced in regular order from before backward there is ground for suspicion that they are intimately connected with the formation of the mesoblastic somites. In view of the fact that the segmentation of the region immediately in front of Groove I is late in

appearing and seldom clearly expressed (Stage 14), we must be on our guard against a possible discontinuity or difference in kind between the segmentation of the anterior and the posterior regions of the cephalic plate. These points can be settled only by a careful study of sections of eggs that have first been described externally; but from surface views alone we are justified in claiming that we have in the open cephalic plate transverse divisions which may be homologized in different embryos, and which are probably of true metameric value; hence they may be of use in solving the vexed problem of the segmentation of the vertebrate head.

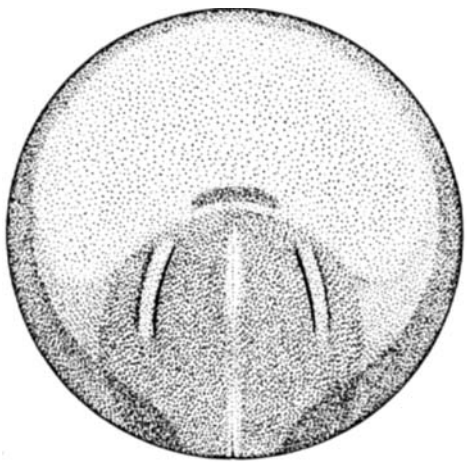


Fig. 159 A living embryo of Cryptobranchus allegheniensis in the early part of Stage 13, viewed in direct sunlight, and so far as possible by transmitted light. From a freehand sketch of the upper hemisphere. × 10.

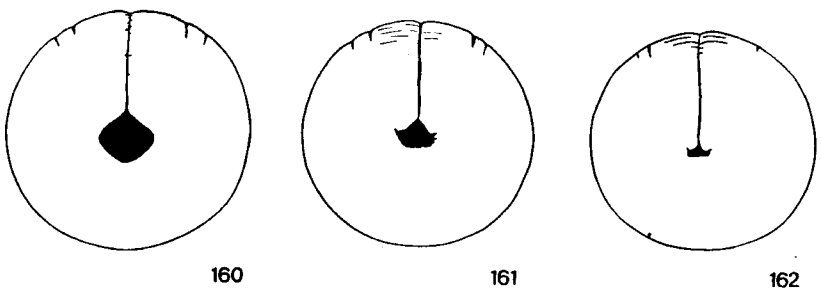
A pair of depressions just within the neural folds near the anterior end of the cephalic plate probably indicate the anlage of the optic vesicles (cf. Eycleshymer '95; Locy, '95).

Some features of this stage are best brought out by the study of living material; for this purpose embryos have been examined in direct sunlight. As shown in figure 159 a transverse opaque band early appears directly in front of the neural plate in the median region; in position and appearance it reminds one of the ectamnion of the chick (Lillie '08, pp. 138 and 139). The neural

folds are conspicuous at an earlier stage in living than in preserved material. In embryos later than the one figured, transverse furrows in the neural plate appear as described in preserved material.

During Stage 13 the blastopore nearly closes, then makes little advance in this respect during the next two stages. Variations in the degree of reduction of the blastopore during these three stages are so great that this structure cannot be used as a character for classifying embryos into stages.

As shown in figures 160 to 162, during Stage 13 the blastopore changes from a diamond shape to that of an anchor; the forward-




Figs. 160 to 162 A series of embryos of Cryptobranchus allegheniensis in Stage 13, showing changes in the size and form of the late blastopore. Camera drawings from preserved material. × 5.

projecting part is derived through an exaggeration of the notch previously noted in the dorsal lip of the blastopore. The lappets lying on each side of this median notch of the blastopore are continuous with the neural folds; through their apposition the dorsal part of the yolk plug becomes closed over. Thus the extreme posterior end of the embryo is undoubtedly formed by a process of concrescence. As shown in later stages, the ventral part of the blastopore becomes reduced to a transverse slit (figs. 177 and 178); during this process the yolk plug usually becomes entirely withdrawn into the egg, but a small mass of yolk sometimes persists at the surface. The late history of the blastopore is much the same in Cryptobranchus japonicus, as described by Ishikawa ('08).

At the close of Stage 13 the neural grove has reached a length of about 124 degrees; its anterior end usually lies quite accurately at the upper vertical pole, while the neural folds extend about 16 degrees in front of it. We have seen that the closing blastopore marks the approximate position of the vegetal pole; this pole has now rotated a total distance of 56 degrees from the vertical axis.

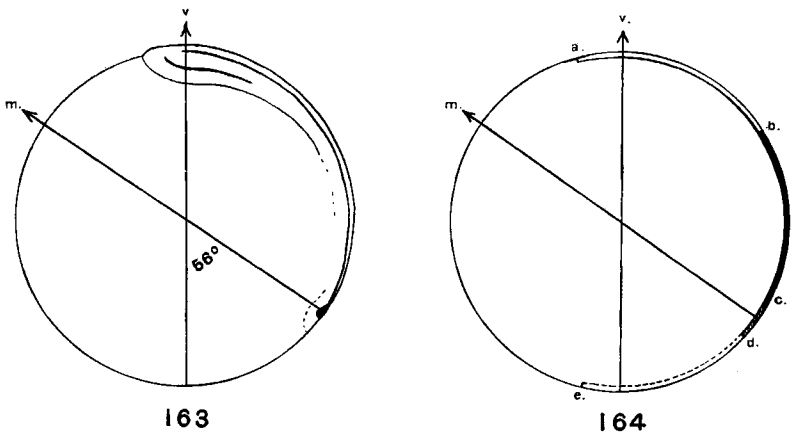



Fig. 163 Diagram of an embryo of Cryptobranchus allegheniensis at the close of Stage 13, showing the position of the neural plate and neural folds with reference to the morphological and the vertical axes; *m*, morphological axis; *v*, vertical axis.

Fig. 164 Diagram showing the position of the embryonic body of Cryptobranchus allegheniensis, and illustrating some features of embryo-formation; *a* to *b* (72 degrees), portion of the embryo formed in situ; *b* to *c* (60 degrees), portion formed by overgrowth of the dorsal lip of the blastopore, with the possibility of concrescence; *c* to *d* (roughly estimated at 16 degrees), portion undoubtedly formed by concrescence; *d* to *e* (60 degrees), distance traveled by the ventral lip of the blastopore. Other lettering as in the preceding figure.

We now have sufficient data for a statement of the position of the embryonic body on the egg, and for pointing out certain features of its mode of formation (figs. 163 and 164). About 72 degrees of the anterior end of the embryo is formed in situ. About 60 degrees is formed in connection with the overgrowth of the dorsal lip of the blastopore; in this case there is the possibility of concrescence through the apposition of material on each

side of the median notch, which may be shifted toward the median line during the process of overgrowth. This point can be definitely settled only by experiment; but in the absence of experimental data we can say that there is no positive evidence of such a process taking place, while certain considerations weigh against it. For in certain observed cases rapid shifting of material is accompanied by a roughening of the surface with the formation of parallel fissures, as in the region just outside of the neural folds during their formation and progress toward the median line. There is an entire absence of any such feature in the dorsal lip of the blastopore.

A region at the posterior end of the embryo, which is roughly estimated at 16 degrees, is formed through the concrescence of the lateral and ventral lips of the blastopore. A part of this material has been brought through a distance of 60 degrees by the overgrowth of the ventral lip of the blastopore; it will be observed that this distance equals that of the overgrowth of the dorsal lip of the blastopore.

At the close of Stage 13, when the embryonic body is for the first time clearly indicated, it has a total length of about 148 degrees. The posterior end is formed around the vegetal pole; the anterior end lies about 40 degrees from the animal pole. Hence the statement made in Part I (Smith '12) to the effect that the axis of polarity of the late ovarian egg defines the principal axis of the embryo is not quite accurate; but the embryo is formed almost wholly in a hemisphere of the egg lying to one side of the axis of polarity. A review of its history shows that the embryo is formed almost entirely out of material derived from a band of cells lying in the equatorial region of the late blastula and early gastrula, and that this band of cells is narrow on the ventral, broad on the dorsal side of the egg (figs. 116 and 123).

Goodale ('11), after reviewing the literature of the subject in connection with his own work on Spelerpes, concluded that

> The amphibian embryo develops almost entirely in a vertical half of the egg, the tail appearing near the lower pole, while the anterior end of the body develops in greater or less degree in the upper hemisphere,

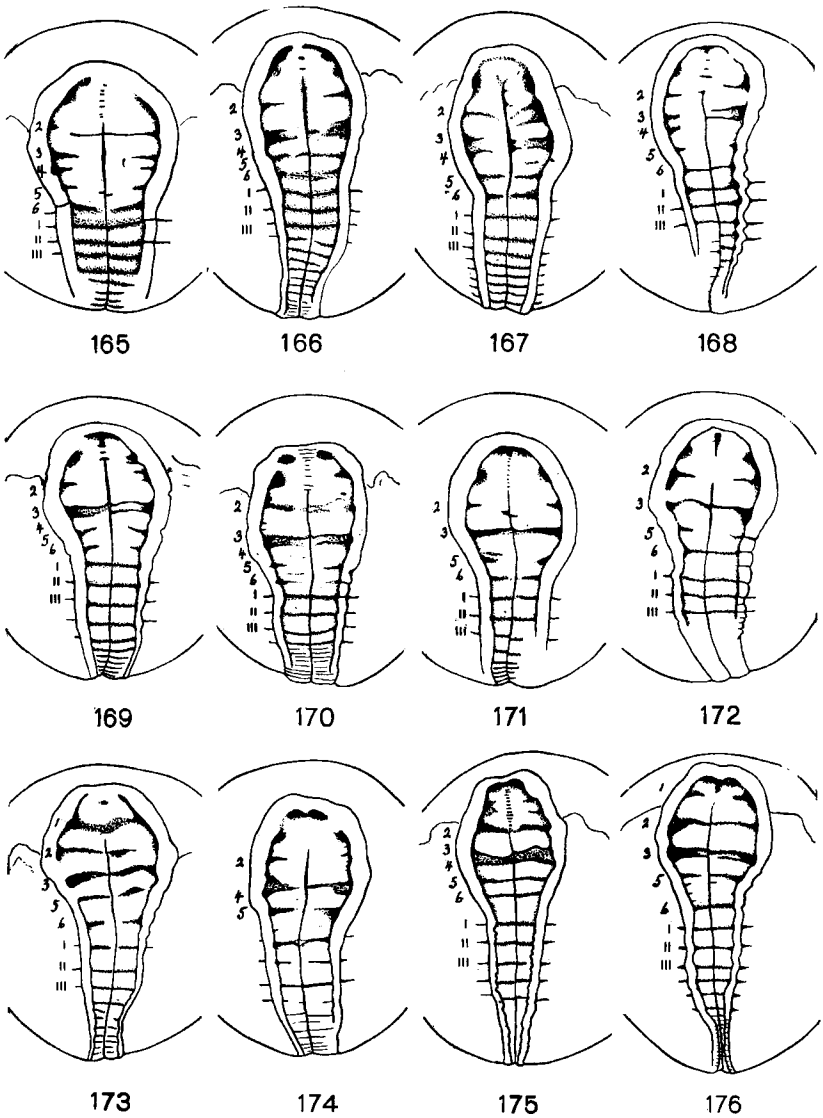
depending upon the particular species. The position of the head of the embryo seems correlated with the length of the embryo, so that the longer the embryo, the higher up on the egg it develops.

The terms 'upper' and 'lower' are evidently here used in the sense of animal and vegetal, that is to say, with reference to those points on the surface of the egg which were in the vertical axis of the egg before it commenced to rotate; therefore the results obtained with Cryptobranchus fall in line with the general statement quoted. The results of Goodale on Spelerpes and my own work on Cryptobranchus agree closely in locating the posterior end of the embryo at the vegetal pole; it is worthy of note that this conclusion was reached independently and by entirely different methods.

*Stage 14: .(figs. 165 to 179 and 229 to 232).* This stage is reached about one day later than the beginning of Stage 13. Since at this time scarcely any two embryos agree in the rate of development of homologous regions of the body, it is impossible in this stage to make a close classification. In marking off this stage from the one following, the principal character considered is the approach of the neural folds toward the median line.

Figures 165 to 176 represent twelve embryos that illustrate the principal changes in the antero-dorsal region during this stage. It will be seen that there is a progressive addition of transverse grooves posterior to the three that first appeared. In front of Groove I the cephalic plate is traversed primarily by six grooves; of these Groove 1, which was noted in the preceding stage, has a very transitory existence and in most cases is lost in Stage 14; likewise the median portion of Groove 2 has often disappeared. Moreover in this or the following stage Groove 4 disappears, following a marked depression and perhaps submergence of the segment between it and Groove 3.

A significant relation exists between Grooves I, II, III, etc., and the intersomitic grooves which now appear just outside the neural folds; by an inspection of figures 165 to 176 it will be seen that in all cases these are in direct apposition. Since the mesoblastic somites are the most characteristically segmented structures of the vertebrate body, it follows that the true segmental units of




Figs. 165 to 176 Antero-dorsal views of embryos of Cryptobranchus alleghe-niensis in Stage 14, showing especially the segmentation of the neural plate. Cam-era drawings finished under the binocular, from preserved material. × 6.

The earliest transverse grooves to cross the neural plate are numbered with Roman numerals in the order of appearance; in front of Groove I the transverse grooves are numbered with Arabic numerals consecutively without regard to the order of appearance. Figure 166 is from the embryo photographed for figures 229 and 230; figure 176 is from the embryo photographed for figures 231 and 232.

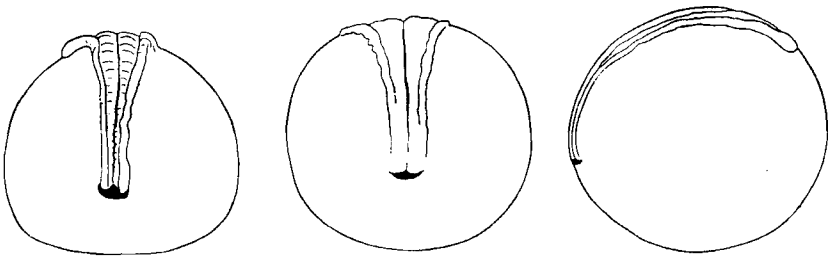
the open neural plate, in this region at least, are the divisions between grooves—that is, the ridges rather than the depressions, for the former are in line with the body somites. If, as appears likely, there is continuity between the structures of the anterior and posterior regions of the cephalic plate, then the rule may be extended to include the entire neural plate. As thus defined, there are seven segments—'neuromeres'—in front of Groove I; posterior to this groove an undetermined number of segments also belong to the head.

In the early stages of the formation of the neural folds transverse grooves are sometimes found in them, continuous with the transverse grooves of the neural plate (see especially figs. 154, 165 and 172). In such cases the neural fold is marked by an outer as well as an inner notch, both in line with the transverse furrow of the neural plate. This condition is only temporary and apparently it is transitional to a later phase in which the inner notch grows at the expense of the outer one, until an outer convexity of the fold appears opposite the inner concavity (see especially fig. 168). In the region of the body somites these outward flexures thus lie in line with the intersomitic grooves as well as with the transverse grooves of the neural plate. This condition is seldom so well expressed as in the embryo shown in figure 168; the convolutions of the neural folds are often irregular and bear no definite relation to the segments. But it is fairly certain that in all cases where the neural folds are well upraised and flexures occur which are segmentally arranged, the outward flexures lie opposite the transverse furrows and not opposite the ridges between them. Moreover, in sagittal sections the transverse grooves on the external surface of the neural plate are found to correspond to ridges on the internal surface.

Those who have described segmental structures in the neural folds or closed neural tube have, as a rule, accepted Orr's ('87) definition of the segmental units or neuromeres as *outward* flexures of the neural folds. But if the above considerations be well founded, the true segments are to be sought rather in the segments between the transverse grooves of the neural plate, and in the *inward* flexures of the neural folds. In other words neu-

romeres are the transverse ridges on the inside rather than on the outside of the brain. To a limited extent this view coincides with that of Kupffer ('85 to '93), who maintained that the true neuromeres are the transverse divisions of the open neural plate rather than the later appearing structures in the neural folds.

Most of the features of this stage thus far described have been observed in living as well as in preserved material. The literature on the early development of the central nervous system has recently been reviewed by Griggs ('10); a more comprehensive



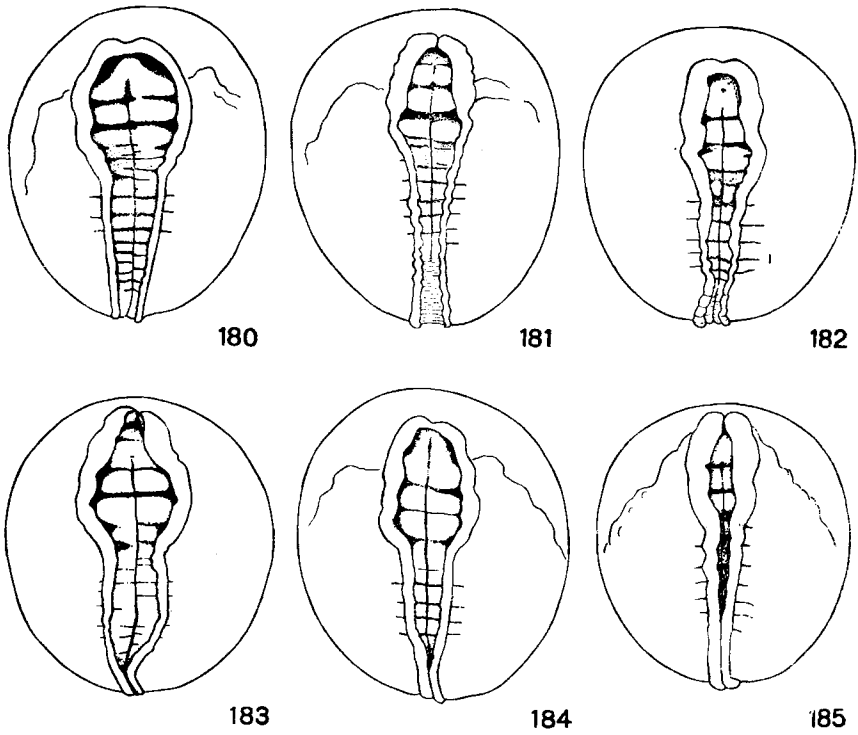

Figs. 177 to 179 Camera outlines of embryos of Cryptobranchus allegheniensis in Stage 14, drawn from preserved material. × 5.

Figs. 177 and 178 Posterior views showing late blastopore.

Fig. 179 Lateral view showing position of the embryonic body at the close of Stage 14. The egg is shown in its natural position with respect to the vertical axis, which passes in the plane of the paper parallel to its lateral margins. The embryo proper has a total length of about 155 degrees. This figure and figure 166 are drawn from the same egg.

survey of the earlier work on the segmentation of the vertebrate head is given by Locy ('95). Ishikawa ('08) has described segmental divisions in the open neural plate of Cryptobranchus japonicus.

During this stage, if not already in the preceding stage, the anterior or dorsal part of the blastopore becomes closed over, while the ventral part persists as a transverse crescentic slit (figs. 177 and 178). At the close of Stage 14 the embryo has increased slightly in length (fig. 179); it now extends over about 155 degrees of the surface of the egg. This increase in length




Figs. 180 to 185 Antero-dorsal views of embryos of Cryptobranchus alleghe-niensis in Stage 15. Camera drawings finished under binocular, from preserved material. Figure 182 is drawn from the embryo photographed for figure 234; figure 184 is drawn from the embryo photographed for figure 233. × 6.

of the embryo involves a noticeable increase in the antero-poste-rior dimension of some of the neuromeres.

*Stage 15: (figs. 180 to 189; 233 and 234).* This stage is reached about eighteen hours later than the beginning of the preceding stage.

In the following account, each neuromere is designated by the number of the groove bounding it on the posterior side. Neuromeres 1 and 2 have usually coalesced; neuromere 4 dis-appears during this, if not in the preceding stage. More definite swellings now occur in neuromeres 1, 2, 3 and 5; the region between Grooves 5 and I is less clearly segmented and is usually somewhat depressed. The outlines of the neural folds in the head region

now suggest the definitive primary divisions (forebrain, midbrain and hindbrain) of the embryonic brain.

The various structures of the neural plate have not yet been followed into the definitive divisions of the embryonic and adult brain; but the preliminary examination of some later embryos dissected by splitting them in the median line with a razor shows that the transverse divisions in the neural plate persist for some time after the closure of the neural folds. Neuromeres in the closed neural tube are also often apparent from the surface. Hence it is easy to judge approximately concerning the fate of individual neuromeres of the cephalic plate, but to avoid possible error it seems best to defer a definite statement until the internal history of the brain has been more carefully studied.

The pair of folds which in the preceding stages extended transversely on each side of the cephalic plate now slant backward (see especially fig. 185); the appearance, particularly in living material, suggests that they are in some way concerned with the origin of the vascular bands which in later stages extend along each side of the yolk sac and give rise to the omphalomesenteric or vitelline veins (fig. 192).

The transverse opacity in front of the neural plate is conspicuous in living material viewed by transmitted light (fig. 186), but is not apparent in surface views of preserved material.

The anterior part of the blastopore is now normally closed over, and the posterior or ventral part is reduced to a transverse slit (figs. 188 and 189). Apparently the middle portion of this transverse slit never becomes completely closed, but in later stages persists as the anal or cloacal opening. The embryonic body has elongated so that it now extends over about half the circumference of the egg (fig. 187).

For the study of transverse divisions in the open neural plate, Necturus is not nearly so favorable as Cryptobranchus. In Necturus the blastopore (figs. 268 to 279) closes much earlier than in Cryptobranchus. Moreover in Necturus the closure of the blastopore is often practically complete; in many specimens preserved at the time of the closure of the neural folds, scarcely more than a vestige of the blastopore is visible from the surface.

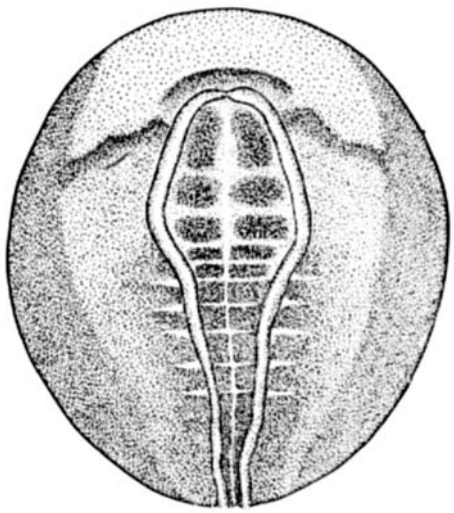


Fig. 186 Antero-dorsal view of a living embryo of Cryptobranchus allegeheniensis in Stage 15, viewed mainly by transmitted light. From a freehand sketch. × 10.

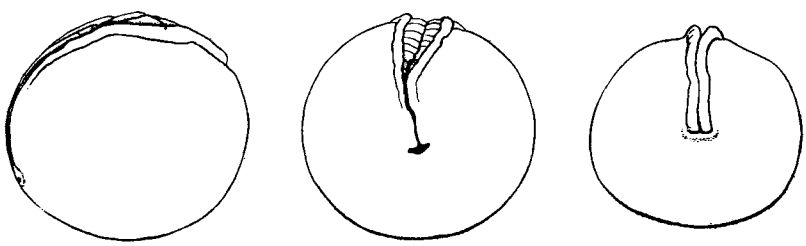



Fig. 187 Lateral view of an embryo of Cryptobranchus allegheniensis in Stage 15, showing the position of the embryonic body. The egg is shown in its natural position with respect to the vertical axis which passes in the plane of the paper parallel to its lateral margins. Camera drawing from preserved material. × 5.

Figs. 188 and 189 Posterior views of embryos of Cryptobranchus allegheniensis in Stage 10, showing the form of the late blastopore. Camera drawings from preserved material. × 5.

Figs. 183, 187 and 188 All drawn from the same embryo.

Figs. 185 and 189 Drawn from the same embryo.

In these later stages, the blastopore is doubtless often indistinguishable in living material (figs. 274 to 276).

A triradiate form of the blastopore is not so frequently found in Necturus; in Cryptobranchus japonicus (Ishikawa '08), it often occurs. A general resemblance may be noted between the blastopore of the urodeles cited and that of the dipnoans (Ceratodus, Semon '01; Protopterus and Lepidosiren, Kerr '09).

### *B. Summary*

Gastrulation involves a combination of the processes of invagination or emboly, and overgrowth or epiboly.

During gastrulation the roof of the segmentation cavity becomes very thin, and is bounded superficially by a sharp furrow, the 'septal furrow' of Ishikawa.

On account of the translucent character of certain parts of the egg, many of the internal changes concerned with gastrulation can be followed quite satisfactorily in living material.

For some time after the beginning of gastrulation, the vegetal pole may be located through the intersection of the first and second cleavage furrows.

During gastrulation and the formation of the neural groove and neural folds the egg rotates on an axis at right angles to the median plane so as to bring the morphological axis at an angle of 56 degrees from the vertical.

The dorsal lip of the blastopore is formed about 68 degrees above the vegetal pole; the ventral lip is formed much later at an equal distance on the other side of the vegetal pole. Since the closing blastopore lies approximately at the vegetal pole, overgrowth proceeds through equal distances on the dorsal and the ventral sides of the egg. During the early part of gastrulation, before the ventral lip is formed, overgrowth takes place rapidly and extensively at the dorsal lip; after the blastopore has become a complete circle, overgrowth takes place very slowly at the dorsal lip, very rapidly at the ventral lip.

Not until after the neural folds are well formed is the yolk plug completely overgrown; as compared with Necturus and

most amphibian eggs the blastopore is very late in closing. In late stages the blastopore has the form of an anchor or an inverted *T*; the posterior transverse portion remains longest as an open slit, and the center of this transverse portion never completely closes but persists as the anal or cloacal aperture.

The posterior end of the embryo forms approximately at the vegetal pole. At the time when the neural folds are first formed the embryo has a total length of about 148 degrees, hence its anterior end does not reach the animal pole. About 72 degrees of the anterior end of the embryo (nearly half its total length) is formed in situ; about 60 degrees posterior to this is formed by overgrowth with the possibility of concrescence. Only a very small part at the posterior end, perhaps 16 degrees, is formed by the meeting of the lateral and ventral lips of the blastopore; this part is undoubtedly formed by concrescence.

From the time of its first appearance the neural groove is continuous with a median notch in the dorsal lip of the blastopore. There is evidence that the neural groove early acquires a segmented structure.

Transverse grooves, definite in number and location, cross the neural plate, dividing it into true segments or neuromeres. In the region of the mesoblastic somites the transverse grooves of the neural plate are in line with the intersomitic grooves, and the neuromeres are in line with the somites. Segmental flexures of the neural folds sometimes occur; in these cases the outward flexures of the neural folds are in line with the transverse grooves, and the inward flexures are in line with the neuromeres.

At the time of the closure of the neural folds, the embryo has increased in length so that it extends over about one-half of the circumference of the egg.

## IX. DEVELOPMENT AFTER THE CLOSURE OF THE NEURAL FOLDS

### *A. Description by stages, to the time of hatching*

Most of the important features of the later external development are sufficiently illustrated by the photographs. Only a brief account is here necessary and this will deal principally with

observations on living material. Some comparisons with Necturus have been given in a previous paper (Smith '11 a). Late stages of Cryptobranchus japonicus have been figured by Ishikawa ('04 and '08) and de Lange ('07).

*Stage 16: (figs. 235 to 237).* This stage is reached about eighteen hours later than the beginning of Stage 15. It is characterized by closed neural folds which are still more or less separated by a median groove. Ganglionic ridges are forming at the sides of the brain. The blastopore is no longer a transverse slit, but a small round orifice which probably represents the definitive cloacal opening. Up to this time the great majority of the eggs have retained the 'vitelline membrane.' During Stage 16 or slightly later this covering usually becomes ruptured as a consequence of the growth of the embryo and is finally cast off.

During the gastrula and open neural groove stages, careful observations have been made to test the presence of cilia on the ectoderm, with absolutely negative results. Currents of water produced by ciliary motion may be detected through the movements of yolk particles within the vitelline membrane when this is present, or by means of powdered carmine added to the water in cases where the vitelline membrane has been shed. At the time when the neural folds are closing, cilia are present on the sides of the body and the ventral surface of the yolk sac, but are absent from the neural folds. The general direction of the ciliary currents is toward the posterior end of the body.

*Stage 17: (figs. 238 to 242).* This stage is reached about a day later than Stage 16. The neural folds are definitely closed and the head well upraised. The optic vesicles are indicated by slight paired expansions of the anterior part of the brain. In some embryos the anlage of the pronephros is apparent through an elevation of the overlying ectoderm. Cilia are absent from the dorsal surface above the neural tube but are quite generally present elsewhere and are particularly strong or numerous on the dorsal surface of the body, lateral to the neural tube. In general the beat of the cilia along the sides and ventral surface of the body is backward.

The embryo is still erect (i.e., with the dorsal surface uppermost). In this position it has been observed, in many cases, to rotate slowly on a vertical axis. To test the direction of rotation a large number of embryos were placed separately in watch glasses and individual records made. Out of sixteen embryos that showed rotation, only two moved in a clockwise direction, the other fourteen in an anti-clockwise direction. The rotation is, of course, caused by the cilia. The direction of rotation in this stage can hardly be explained as the result of a tendency for the embryos to lean to one side oftener than to the other, for, as will be shown in a later stage, the facts are otherwise. Possibly more extended observations would show more equality in the results; or there may be a uniform asymmetry in the distribution, or in the rate or direction of beating, of the cilia of the ventral surface of the yolk sac.

*Stage 18: (figs. 243 and 244).* This stage is reached about twenty-four hours after the beginning of Stage 17. It is characterized by a prominent outstanding head with marked cephalic flexure and distinct optic vesicles, and by the presence of the pronephros and the first definite indications of the budding tail. During the latter part of this stage the mandibular arch is usually recognizable.

Patches of cilia are now distributed over the entire surface; the beat of the cilia is in general backward and the currents are much the same as figured in the next stage (fig. 190).

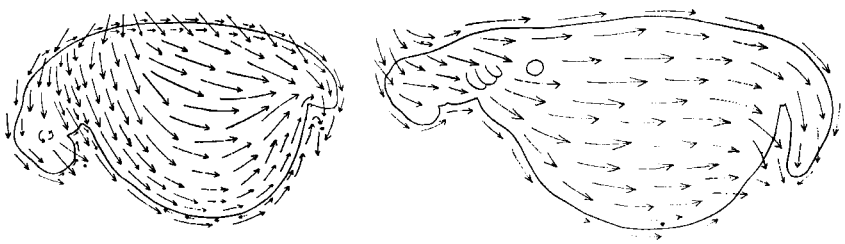
During this stage the embryo topples over from its erect position so as to fall to one side, on which it lies throughout several succeeding stages until spontaneous movements enable it to change its position. An incidental result of this position is to bring a larger area of the ciliated surface into contact with the substratum; as a consequence of this and of the stronger ciliation, rotation of the embryo is now of more marked occurrence. The most rapid motion observed was performed by an embryo that completed a rotation in just two minutes.

The functional value of the ciliary motion is at least two-fold: (1) it bathes the surface of the embryo with currents of water which are subservient to respiration; and (2) rotation of the

embryo, when it occurs, serves to prevent adhesion of the embryo to the envelope with consequent abnormalities.

The ciliation and rotation of the frog embryo have been described by various writers, notably Assheton ('96). Piersol ('09) has described rotation in the embryo of Plethodon.

*Stage 19: (figs. 190 and 245 to 248).* This stage begins about twenty-four hours later than Stage 18. It is characterized by from two to three distinct gill invaginations, a budding tail, a very marked outward expression of the pronephros (see especially figs. 245 and 246), and beginning lateral vascular bands, the anlage of the vitelline veins (see especially fig. 245). In addition



190 191

Fig. 190 Diagram of an embryo of Cryptobranchus allegheniensis in Stage 19, showing the direction of the water currents produced by cilia.

Fig. 191 Same as figure 190, for Stage 21.

to the cephalic flexure there is a slight cervical flexure which reaches its maximum in this stage. About sixteen to twenty mesoblastic somites are apparent in surface views.

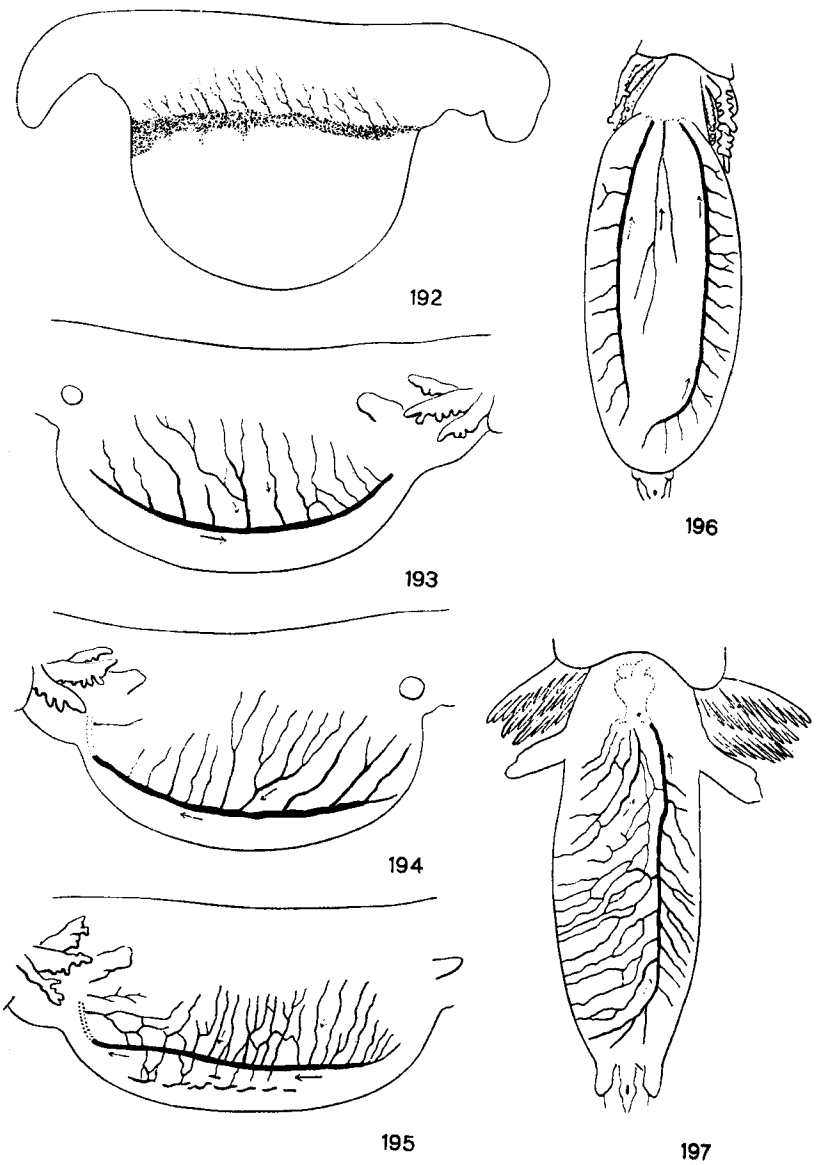
In the living embryo, the lateral vascular bands are conspicuous structures, for they are pink with blood; but they are not yet differentiated into true veins. Overlying the upper part of the yolk sac, they extend from the heart region longitudinally on each side of the body and meet posteriorly a little below the tail. During this stage and the stages immediately following, they shift slowly toward the ventral surface. There is considerable variation in the position of this band in embryos that are otherwise in the same stage. A similar vascular area has been figured for Cryptobranchus japonicus by de Lange ('07).

In this stage practically all embryos are found lying on either the right or the left side. To determine the relative number of cases of each, one hundred and nine embryos, taken at random from several different spawnings, were examined; fifty were found to lie upon the right side, fifty-nine upon the left side. These numbers are approximately equal, hence the occurrence follows the laws of chance.

Rotation of the embryo now occurs in a greater proportion of cases than in the preceding stage, though of the embryos studied hardly more than one in ten has been observed to rotate. It is noticeable that in some cases the direction of rotation is clockwise, in other cases anti-clockwise. In eleven embryos that showed rotation eight moved in an anti-clockwise direction; all these were lying on the left side. Three embryos rotated in a clockwise direction; of these two were lying on the right side, one on the left side. Hence as a general rule the direction of rotation is correlated with the position of the body. The actual direction is not what one would expect if the beat of the cilia were directly backward in all parts of the body; but by an inspection of figure 190 it will be seen that in the head region the beat is ventrad, in the posterior region dorsad. The ciliary currents are alike on the two sides of the body.

*Stage 20: (figs. 192 and 249 to 251).* This stage is reached about two days later than the beginning of Stage 19. Through the loss of the cervical flexure the head is now brought more nearly in line with the body, but on the other hand there is an increase in the cephalic flexure. Five gill invaginations are usually visible, the two posterior ones being sometimes indistinct. There are distinct nasal pits. The roof of the medulla is becoming thin and transparent. From twenty-five to thirty mesoblastic somites are apparent in surface views. The tail undergoes a decided increase in size, and is slightly flexed ventrally. The front limb anlagen appear during the latter part of this stage.

The lateral vascular bands are not yet differentiated into veins, but during the latter part of this stage some small veins have been observed, in living material, extending vertically from the vascular bands for a short distance above them (fig. 192). No




Figs. 192 to 197 Development of the vitelline veins of Cryptobranchus allegheniensis. All the figures are from living material and, with the exception of the veins in figure 192, are drawn with the aid of a camera. × 5.

Fig. 192 Stage 20. The stippled area indicates the lateral vascular band. The right and left sides of the embryo are practically alike.

Figs. 193 and 194 Right and left views of an embryo in Stage 22.

Fig. 195 Lateral, slightly ventral, view of an embryo nearly ready to hatch.

Fig. 196 Ventral view of an embryo nearly ready to hatch.

Fig. 197 Ventral view of a larva about ten days after hatching.

movement of the blood has been observed in this stage. The embryos are ciliated, and undergo rotation, much as in the preceding stage. Muscular movements do not ordinarily occur, but have been observed when the embryos were placed in fixing fluids.

*Stage 21: (figs. 191 and 252 to 255).* This stage is reached about four days later than the beginning of Stage 20. It is characterized by the presence of three budding external gills, and small front limb rudiments. The tail is longer, and has a more decided ventral flexure, than in the preceding stage.

The dorsal surface of the embryo is now for the first time slightly pigmented. The pigmentation begins in that portion of the ectoderm overlying the nervous system, and gradually extends downwards over the sides of the body. During this and the following stages (McGregor '97), the pigment cells in the region of the mesoblastic somites are grouped metamerically.

Numerous veins now branch off dorsally from the lateral vascular bands. In the bands themselves, the two main trunks of the omphalo-mesenteric or vitelline veins, one on each side of the yolk sac, are being differentiated, but they rarely become complete before the next stage. In general the differentiation of the vitelline veins has gone further on the side of the body that happens to be uppermost. During the latter part of this stage the heart is pulsating regularly, with about twenty-five to forty beats per minute; the blood sometimes surges back and forth in the principal veins overlying the yolk sac, with a slight excess in the forward movement, but the vitelline circulation is not yet completely established.

The distribution of cilia remains much the same as in the preceding two stages, but the ciliary currents move more uniformly toward the posterior end of the body (fig. 191).

Spontaneous muscular movements, consisting chiefly of a bending of the body laterally into a *U* shape, now occur, but the embryo is as yet unable to turn over.

*Stage 22: (figs. 193, 194 and 256 to 259).* This stage begins about five days later than Stage 21; its most distinctive characteristic is that the external gills have rudimentary branches and

are pink with blood. The tail is rapidly increasing in size and beginning to straighten out. The front limb rudiments now take the form of conspicuous outstanding lobes, the distal ends not yet divided into digits. Toward the close of this stage the rudiments of the hind limbs appear. The entire dorsal surface of the body is well sprinkled with pigment cells; the eyes are becoming deeply pigmented. The lateral line system is well developed. During this stage occurs the rapid formation of the gular folds.

The lateral vascular bands no longer appear as such, but on their site are differentiated the two main trunks of the vitelline veins (figs. 193 and 194). As compared with the earliest position of the lateral vascular bands, the vitelline veins lie considerably nearer the ventral surface of the yolk sac. While the vitelline system of veins is primarily a paired one, almost from the beginning one side is usually found better developed than the other. The heart now contracts at the rate of about forty to sixty beats per minute, and the blood pulsates regularly through the vitelline veins.

The cilia are especially well developed on the external gills; here the ciliary currents are strongest. Spontaneous muscular movements now occur at frequent intervals. The movements consist of jerking the head from side to side; wriggling; reversal of the laterally curved position of the body by turning over; swimming movements by means of which the embryo butts against the envelope; and swimming in a circle. The functional value of these movements seems to be to afford exercise for the developing muscles. Embryos removed from the capsules at this stage make practically the same movements; they are unable to progress in a straight line and are incapable of prolonged swimming movements.

During the later stages of development before hatching, the water in which the embryos are kept has a pronounced 'fishy' odor.

*Stage 23: (figs. 195, 196, 260 and 261).* The limits of this stage are fixed to include the time of hatching. In a given lot of embryos hatching does not take place all at once, but extends

over a period of about a week, with a corresponding variation in the degree of development of different individuals at the time of escape from the envelopes. On the average, hatching occurs about two weeks later than the beginning of Stage 22, and about six weeks after fertilization. Previous to the hatching period, the envelopes become much softened and considerably enlarged by the absorption of water, making room for the growing embryo. The latter usually escapes by pushing ('worming') its way through the envelope, leaving a small round hole; in some cases it bursts the envelope by means of wriggling movements.

The newly hatched larva measures about 23 to 25 mm. in length. Very noticeable is the retention of a large yolk sac with conspicuous bright-red vitelline veins; the bushy external gills are pink with blood. In proportion to size of body, the tail is much larger than in the adult. The dorsal surface of the body and the sides of the tail are well pigmented, but in general the larva of Cryptobranchus, like that of Necturus, is pale as compared with amphibian larvae that develop from a pigmented egg exposed to the light. The ventral surface is lacking in pigment, leaving the abdominal region yellow from the presence of yolk, and the throat region transparent. The heart can be readily observed without dissection. The anterior limb rudiment is provided with two digits. In most specimens the body somites are plainly visible, but they do not show well in the photographs. On account of its large size, graceful outlines and bright colors, the newly-hatched larva is a striking and beautiful object.

In the resting position, the larva lies on its side, turning occasionally from one side to the other. The newly hatched larva is able to swim rapidly in a straight line for a short distance, using the tail as a propeller. The larvae avoid the light, and are positively rheotactic.

The vitelline veins (figs. 195, 196 and 261) have shifted further toward the mid-ventral line; on one side of the body these veins are well developed, on the other side they show arrest of development with signs of atrophy. The heart now beats about sixty to seventy times per minute.

Aëration of the blood is afforded, not only by the external gills, but by the capillaries lying close to the surface over all the body. On account of its great exposed surface, the tail may be of especial importance as a respiratory organ.

As in the preceding stage, cilia persist over the entire surface of the body, and the ciliary currents are strongest in the vicinity of the gills.

### *B. Larval development, and the metamorphosis*

The changes in the form of the body, and the gradual increase in size, during the first year of larval development, are shown by the photographs (figs. 262 to 267).

Year-old larvae reared in the laboratory reach a length of about 5 to 7 cm.; three two-year-old specimens reared in the laboratory measured respectively, after preservation in alcohol, 7 cm., 8 cm. and 9.5 cm. Near the close of the second summer these latter specimens lost their external gills. The few specimens with external gills taken in August from their natural environment (they were found under small flat stones in shallow water) measured as follows: 6.4 cm., 6.8 cm., 7.0 cm., 7.3 cm., 7.7 cm., 12.0 cm., 12.3 cm. It will be noticed that these specimens sort into two lots, one lot containing those with body lengths ranging between 6 and 8 cm., the other lot containing specimens approximately 12 cm. in length. Though this data is rather meager, the rather considerable gap between the two lots suggests that we are dealing with larvae of the first and second summers, respectively. In comparing the larger larvae taken from their natural environment with the two-year-old specimens reared in the laboratory, allowance must be made for the fact that the latter were measured after being shrunken by preservation in alcohol; moreover the body form of the laboratory specimens seems to be shorter and stouter than the normal. Specimens with a body length of 14 cm. and more, taken from their natural environment, have invariably lost their external gills. The combined evidence from specimens reared in the laboratory and

those taken from their natural habitat indicates that the metamorphosis occurs at the end of the second year.

At the time of hatching, the embryo retains a supply of yolk sufficient to last it for several months; the mouth is still quite ventrally situated. So far as its method of nutrition is concerned, during this period the young Cryptobranchus is an embryo rather than a larva. Gradually the yolk disappears, and the mouth assumes a terminal position. Specimens reared in the laboratory begin to take food about two to four months after hatching; they must be fed individually with bits of scraped beef. No notice is ordinarily taken of the food unless it is moved about immediately in front of the animal and preferably a little to one side of the mouth. Some of the specimens take food more readily, and grow more rapidly, than others. One lot of larvae, reared in the laboratory, ate young frog tadpoles. A 12 cm. specimen taken from its natural habitat ate a large Corydalis larva; another newly captured 12 cm. specimen regurgitated a partly digested 6 cm. larva of its own kind.

During the first month after hatching, the vitelline veins of one side of the yolk sac degenerate, while those of the other side shift to a more nearly median position (fig. 197). Degeneration of the right or the left vitelline vein takes place in about an equal number of cases. It has already been noted that in those stages when the embryo lies continuously on one side the vitelline veins are best developed on the uppermost side; furthermore that the embryo falls on the right or the left side in about an equal number of cases. The facts strongly suggest that the position of the embryo during the period when the vitelline veins are developing is the factor that determines on which side the vitelline vein shall persist; but since making these observations I have had no opportunity to put the matter to a rigid test.

With the reduction of the yolk sac, the vitelline circulation suffers a corresponding diminution in extent; during the late stages of this process, through the increasing thickness and opacity of the ventral body wall the vitelline veins are somewhat obscured.

In the free-swimming larva, the heart beats more rapidly than was the case during embryonic development.

Within a week or two after hatching, the rapid growth of the front limb rudiments enables the larva to support itself in the normal position of the adult. One month after hatching, the front limbs have increased decidedly in length and possess the full number of digits (four). The form and position of the front limbs adapt them for use as paddles; by means of a simultaneous backward stroke they aid the larva in getting a quick start for swimming. The posterior limbs develop more slowly; at this time they are relatively short and as a rule possess but three digits, though in some cases the full number (five) are present. Ten weeks after hatching in all cases both pairs of limbs possess the full number of digits and are used in walking in the same manner as in the adult. The limbs are broad and flat, and in swimming at a moderate rate of speed are used as paddles. After the sixth month of larval development the posterior limbs surpass the anterior in size and strength. In the two-year-old specimens reared in the laboratory the limbs appeared weak and poorly developed as compared with newly captured specimens of the same age.

As in the adult, the tail is the principal organ of locomotion during rapid swimming. Up to five or six months after hatching the tail remains much larger in proportion to body-size than in the adult. In the year-old larva the tail is proportionally much smaller than in specimens ten weeks after hatching. In the two-year-old specimens reared in the laboratory the tail is smaller than in newly-captured specimens of the same age.

One month after hatching, pigmentation is greatly advanced and extends over the external gills; when viewed from above the larva is now nearly black. The ventral surface remains white and nearly transparent in the throat region, yellow in the region of the yolk sac. Six months after hatching, the dorsal surface shows large dark spots of unusually dense pigment, which are characteristic of all the later stages; the ventral surface is slightly pigmented. In the year-old larva, the dorsal surface is still nearly black, but with a few scattering inconspicuous yellow spots; the abdomen is grayish and the throat region almost

white. In the two-year-old larva the general color effect is not so dark; the larva is taking on the variegated color pattern of the post-larval and adult stages.

In the month-old larva, shedding of the cuticle was observed for the first time. From this time on, the water of the aquarium becomes almost cloudy with detached portions of epidermis.

Soon after the hatching period, cilia disappear from the general surface of the body, but persist on the gills where a strong eddying current of water is produced. The gills remain ciliated until at least six months after the hatching period; at this point observations were necessarily discontinued.

As early as five months after hatching and frequently thereafter, larvae have been observed to come to the surface for air and to give up large bubbles of air from the mouth, indicating that the lungs are functional.

During the first year of larval life, the ventral portions of the three gill arches bearing the external gills are expanded into leaf-like plates, partly covered by the opercular lateral portions of the gular fold. At this time there are three gill openings. Toward the end of the first year the median portion of the gular fold disappears, but the lateral portion extends as a small opercular fold above the root of the anterior external gill. During the second summer the opercular fold extends dorsally far enough to cover the bases of all three external gills. With the loss of the external gills the opercular flap persists and partly roofs over a shallow cavity containing the leaf-like plates previously mentioned.

### *C. Post-larval stages*

Sexual maturity is attained with a length of at least 30 cm. for the male, and 35 cm. for the female. During August, specimens 14 to 20 cm. in length have been very rarely taken, while specimens measuring from 20 cm. upwards are very plentiful; this suggests that the young ordinarily reach a length of at least 20 cm. at the end of the third year, and that they do not ordinarily become sexually mature until the end of the fourth year.

The immature post-larval stages resemble in coloration the young adults described in Part I. Aside from the loss of the external gills, the most conspicuous changes as compared with the larvae are a slight progressive dorso-ventral flattening of the body, and the gradual development of the folds of the skin which are so prominent in adult and especially in very large and presumably old specimens. A transverse slit-like spiracular opening is bordered anteriorly by the small opercular flap and posteriorly by a similar but still smaller fold of skin. The spiracle leads to a small cavity containing a single persistent gill opening bordered by two leaf-like plates; the other gill-lamella is greatly reduced or has disappeared.

### *D. Summary*

The vitelline membrane is shed about the time of the closure of the neural folds.

Shortly after the closure of the neural folds, the entire surface of the embryo becomes ciliated. The beat of the cilia is in general toward the posterior end of the body; currents of water are produced which are subservient to respiration. After the appearance of the external gills, ciliary currents are especially strong in their immediate vicinity. Soon after the hatching period, the cilia disappear except on the external gills; here they persist until at least six months later.

Soon after the closure of the neural folds, the embryo falls on one side, where it lies until, in a much later stage, it is able to turn over through muscular activity. The embryo falls on the right or the left side in about an equal number of cases.

Rotation of the embryo, due to the beating of the cilia, commences before the embryo has fallen on its side but is more pronounced afterward. In most cases rotation proceeds in a clockwise direction when an embryo is lying on its right side, in an anti-clockwise direction when it is lying on its left side. Rotation may be of service in preventing adhesion of the embryo to the capsule.

The vitelline veins develop as paired structures along the sides of the yolk sac, and shift gradually toward the ventral

median line. The vitelline veins develop more rapidly on that side of the yolk sac which happens to be uppermost. Soon after the hatching period, the vitelline veins of one side degenerate, while those of the other side reach their fullest development; the veins of the right or the left side persist in about an equal number of cases. Probably the position of the embryo during the stages when it lies continuously on one side is the factor that determines which set of vitelline veins shall gain the ascendancy.

The newly hatched larva retains a supply of yolk sufficient to last it from two to four months.

The tail of the early larva is proportionally much larger than in the adult.

Pulmonary respiration is established about five months after the hatching period.

The metamorphosis takes place at the end of the second year.

Sexual maturity is attained, probably at the end of the fourth year, with a body length of at least 30 cm. for the male and 35 cm. for the female.

## X. TIME RECORD

For the characteristics of the different stages reference is made to the text and illustrations, especially the photographs. For methods used in obtaining the time record, see the introduction (Section VI). Table 1, page 534.

## XI. ABNORMALITIES

The present section deals primarily with abnormalities found in embryos taken from their natural environment, or kept under conditions as nearly normal as possible.

### *1. Large yolk plug*

In the early stages of development the most common abnormality is the presence of an unusually large yolk plug. Examples are shown in figures 198 and 199, though these are far from representing extreme cases. In well-marked examples the blastopore forms a complete circle only a little below the equator, and

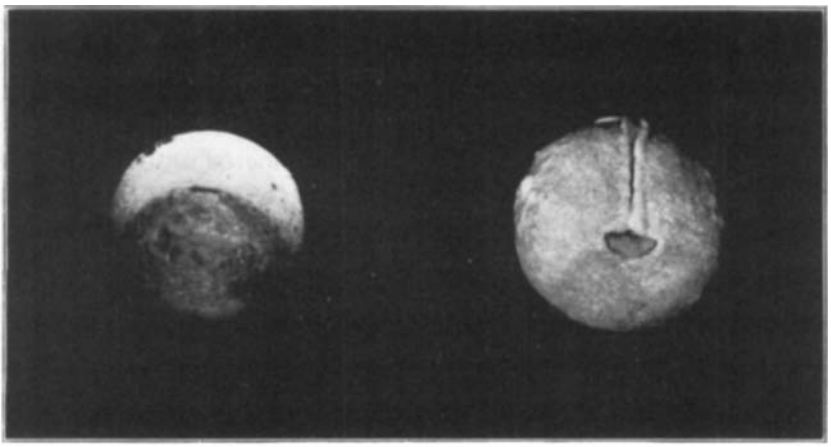
TABLE 1

| STAGE | TIME AFTER FERTILIZATION | INTERVAL |
|---|---|---|
| 1 | 24 hours (18 to 28) | |
| 2 | 30 hours (24 to 36) | 6 hours (5 to 7) |
| 3 | 35 hours (29 to 44) | 5 hours (4 to 6) |
| 4 | 39 hours | 4 hours (3 to 5) |
| 5 | 43 hours | 4 hours |
| 6 | 47 hours | 4 hours |
| 7 | 51 hours | 4 hours |
| 8 | 63 hours | 12 hours |
| 9 | 3½ days | 19 hours (14 to 24) |
| 10 | 5 days | 36 hours (24 to 48) |
| 11 | 7 days (6 to 8) | 48 hours |
| 12 | 10 days | 72 hours |
| 13 | 11½ days | 36 hours |
| 14 | 12½ days | 24 hours |
| 15 | 13¼ days | 18 hours |
| 16 | 14 days | 18 hours |
| 17 | 15 days | 24 hours |
| 18 | 16 days | 24 hours |
| 19 | 17 days | 24 hours |
| 20 | 19 days | 48 hours |
| 21 | 23 days | 4 days |
| 22 | 4 weeks | 5 days |
| 23 | 6 weeks | 2 weeks |

Metamorphosis: Two years after fertilization.

a large yolk plug persists long after the closure of the neural folds; such embryos usually produce normal larvae, though spina bifida is an occasional result. In extreme cases the blastopore may form entirely above the equator; such embryos die before reaching the larval stage.

This abnormality appears most frequently in eggs that have been kept in jars of water during warm weather, and especially in material that has been shipped long distances. Probably it may be brought about by a variety of unfavorable conditions: heat, lack of oxygen, mechanical agitation, and injurious substances (e.g., cinders) in the water. It is readily produced by treatment with sodium chlorid; very marked cases of spina bifida may result.



198 199

Figs. 198 Postero-dorsal view of a gastrula of Cryptobranchus allegheniensis with an unusually large yolk plug. The blastopore extends around the egg as a complete circle, but only the dorsal portion shows distinctly in the photograph. From preserved material. × 4.

Fig. 199 Posterior view of an embryo shortly before the closure of the neural folds, showing persistent yolk plug. Photographed from preserved material. × 4.

### *2. Exovate abnormality*

A rather common abnormality originating at the time of the formation of the neural groove may be called the exovate abnormality. A small protuberance on the site of the closed fenestra takes the form of a spherical exovate; in some cases this reaches a diameter about half as great as that of the egg. In most cases the extra-ovate remains connected with the egg by a very narrow stalk; the malformation is entirely extra-embryonic, the protruded yolk is gradually absorbed and the egg produces a normal larva. In other cases the protuberance takes the form of a dome-like swelling and increases in size until the egg collapses.

### *3. A double embryo*

On September 27, 1906, I found a nest containing embryos in an advanced stage of development, and among them the double

embryo shown in figure 200. The total bulk of this double embryo is about equal to that of an ordinary single embryo from the same spawning. That the occurence of such a monstrosity in this species is exceedingly rare in nature is shown by the fact that during the past seven years I have collected many thousands of embryos from nests, yet in no other instance have I found an abnormality even approaching the one under consideration.

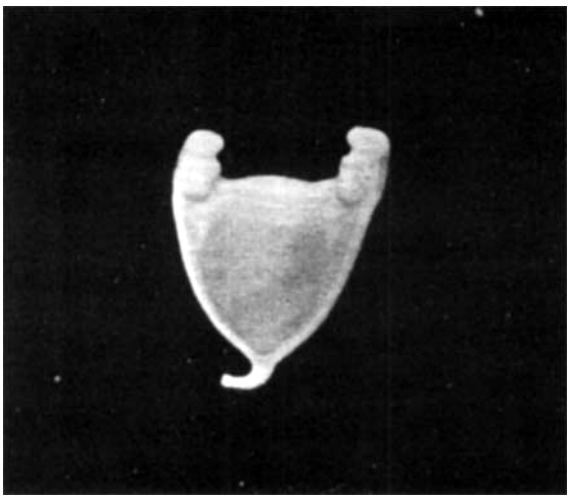


Fig. 200 Double embryo of Cryptobranchus allegheniensis. Photographed after preservation in formalin. × 4.

The precise manner in which the double embryo originated is problematical. Single capsules sometimes contain two eggs in close contact; but the small size of the double embryo precludes the possibility that it was formed by the union of two such eggs. Through treatment with sodium chloride I have produced embryos in which the brain differentiated without closure of the neural folds, but no true double embryos were obtained by this means; moreover in nature it is improbable that unusual chemical influences should affect a single egg.

It is more likely that the abnormality was brought about by mechanical means. A partial separation of the first two blasto-

meres (e.g., through the egg becoming lodged in a crevice) might in some cases result in the formation of a double embryo (Spemann '01 to '03). But owing perhaps to the heavily yolk-laden character of the egg, and the slowness with which the first furrow cuts its way through the yolk, my attempts to produce a double embryo in this manner have failed. When a fine silk thread or a hair was tied about the egg in the two-cell stage so as to constrict it in the plane of the first cleavage furrow, the egg usually burst before reaching the gastrula stage. In a single case the egg lived until after the formation of the neural folds, but developed a single embryo with its principal axis at right angles to the constricting cord.

A more probable explanation is that in the two-cell stage the egg became inverted and remained for some time in this position subject to the rearrangement of contents through the disturbing influence of gravity acting in a direction opposite to the normal (Schultze '95). But the whole question is complicated by the more fundamental problem of the determination of the median plane of the embryo. My experiments, to be described in a later paper, show that in Cryptobranchus the first cleavage furrow tends to form at right angles to the direction of entrance of the sperm (as in Triton, but not as in the frog where the first cleavage tends to coincide in direction with the path of the sperm). If in Cryptobranchus, as in the frog, the entrance path of the sperm lies approximately in the median plane of the embryo, then the conditions necessary for the production of a double embryo by the separation of the first two blastomeres must be of very exceptional occurrence.

### *4. Spiral-tailed monsters*

In the fall of 1910, some embryos shipped by express to the University of Wisconsin and reared there in city water, acquired, in about 8 per cent of their number, the abnormality shown in figures 201 and 202. From the condition of the tail in late larval stages, specimens affected with this malformation may be designated as 'spiral-tailed monsters.' At first this peculiarity seemed

merely the persistence of an embryonic condition; for in early stages the tail is always strongly flexed and in these particular cases it failed to straighten out. But as development progressed the tail became twisted into a pronounced spiral. The illustrations show the extreme condition; cases occur forming a series intermediate between this and the normal. In some cases the back is arched or humped. So far as can be judged from surface views,

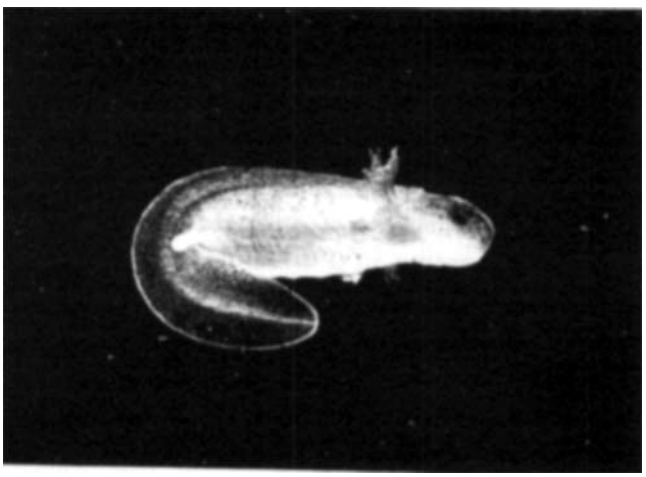


Fig. 201 Spiral-tailed monster of Cryptobranchus allegheniensis at the time of hatching. Photographed from the living embryo anaesthetized with chloretone. × 3.

the entire abnormality seems to be brought about by the constricting effect of a band of tissue lying in the ventral median line.

As a result of this malformation, the larva is compelled to lie on one side, and can swim only in a grotesque fashion, with backward circling movements. Affected larvae take no food, and die shortly after using up their supply of yolk.

An analysis of the water was secured but the subject has not yet been further investigated. Stockard ('06, p. 119) noted the occurrence of a similar abnormality in Fundulus embryos.

*5. Critical periods in the life history*

In closing this section it remains to note that there are three stages in the life history characterized by unusual mortality—nature's examinations occur in these stages. These critical periods of development are:

Fig. 202 Spiral-tailed monster of Cryptobranchus allegheniensis two months after hatching. Photographed from the living larva anaesthetized with chloretone. × 3.

(*a*) *The late blastula.* Many eggs seem unable to form a gastrula.

(*b*) *The hatching period.* Many embryos seem to lack the strength necessary to escape from the gelatinuous envelope, and die in the capsule.

(*c*) *The period of change in the method of nutrition.* After exhausting their supply of yolk, many larvae refuse to take food, or seem unable to set up digestive processes, and die of starvation.

## XII. PHYLOGENY

We are here concerned with (a) the origin of the amphibia in general, (b) the origin of the urodeles, and (c) the interrelationships of the urodeles with special reference to Cryptobranchus. The evidence may be classified as (*a*) anatomical, (*b*) paleontological, and (*c*) embryological. In the following survey, no attempt is made to keep these lines of evidence strictly separate; for paleontology is simply an extension of comparative anatomy to fossil forms, with the added element of sequence in time; while the development during the late embryonic and larval stages gives the key to many adult structures that could not otherwise be homologized.

It is not within the scope of this paper to enter extensively into anatomical and paleontological questions; but as a check on possible generalizations derived from the study of amphibian embryology the writer has devoted considerable time to a study of the paleontological material in the American Museum of Natural History, and has endeavored to acquire some degree of familiarity with the results of modern research in this field. In this work he has been greatly aided by Dr. W. K. Gregory, who has in progress a detailed review of the origin of the amphibia (see abstracts in Science, Gregory '11 a, '11 b and '12).

The tetrapoda form a coherent group. The gap between this group and the fishes is one of the weak places in vertebrate phylogeny, and it is here that the idea of continuity in the descent of the higher vertebrates has been most often attacked; some have maintained a diphyletic origin for the tetrapods and fishes.

The amphibia undoubtedly include the most primitive known tetrapods. It is on this account that the amphibia possess a peculiar interest from a phylogenetic point of view; the problem of the origin of the amphibia is the problem of the origin of the tetrapods in general.

The fundamental unity of the gnathostome type leads us to look among the true fishes for the nearest living or fossil representatives of the ancestral stock of the amphibia. For in fishes and tetrapods the following features, as well as many others,

are homologous: the chief divisions of the brain; the cranial nerves; the eye muscles and their innervation; the chondrocranium arising from trabeculae and parachordals, and including olfactory, optic and auditory capsules; the visceral arches; and the essential structures of the circulatory system. If we compare only the amphibia with the fishes the range of resemblances becomes still greater; all the important structures are in essential agreement except those concerned with the outer halves of the paired extremities.

The argument for a diphyletic origin of the pisces and amphibia is based upon (*a*) the lack of homology in certain elements of the skull, and (*b*) the difficulty that is experienced in deriving the amphibian limb from the fin of any known fish. But the resemblances in the skull bones become very close when we consider fossil forms, and the trend of increasing knowledge is in the direction of more complete homology rather than the reverse. Moreover we find difficulties almost as great in homologizing cranial elements in different fishes; for these structures are plastic and exposed to environmental influences, and with the radical change from an aquatic to a terrestrial habitat we should expect them to be profoundly influenced. The endoskeleton of the paired appendages presents us with a problem of greater difficulty but here, too, we have to deal with structures that we should expect to be greatly modified in connection with the change of habitat. In view of the wide range of resemblances in important structures one is hardly inclined to consider seriously the idea of a diphyletic origin for the fishes and amphibia.

The amphibians must have descended from some fish having scales with the potentiality of fusing into bony plates. The dermal bones forming the roof of the skull must have been arranged in pairs on each side of a median suture; for this is the condition found in the most primitive known amphibians (e.g., Branchiosaurus). The ancestral form must be sought in some fish having the endoskeleton of the paired fins widely protruded from the body, and with pectoral and pelvic members similar. Such fins functioned primitively as paddles; with the adoption of a terrestrial method of locomotion, by creeping or crawling on a

more or less solid substratum, the endoskeletal elements of the limbs become greatly strengthened and progressively longer in order to lift the body from the ground. Such a progressive elongation of the limb bones, particularly the proximal elements, may be traced in both fossil fishes and amphibians; in the latter the pelvic girdle is also found becoming definitely articulated with the axial skeleton. The ancestral form must have been short-bodied; for in many groups of animals a progressive elongation of the body, culminating in eel-like forms, is found to accompany a degenerate structure scarcely capable of giving rise to higher forms (Gregory '07, Appendix I).

The lack of scales with the potentiality of fusing into bony plates is alone sufficient to exclude the elasmobranchs from the immediate ancestry. For affinities ancestral to the amphibia most authors have looked to the crossopterygii or the dipnoi. Both have dermal bones, and both fulfil the requirement regarding fins with widely protruded basal lobes and with endoskeletal elements from which the framework of true limbs might be derived.

At first sight the dipnoi seem best to bridge the gap between fishes and amphibia. For the lung-fishes have survived by virtue of an approach to the tetrapod type, enabling them to exist during periods of drought. But various considerations derived from the study of paleontology and comparative anatomy make it probable that these terrestrial adaptations were independently acquired, and that the dipnoi were already too highly specialized in other respects to give rise to the amphibia. The mosaic of small bones forming the greater part of the roof of the skull, particularly in the fossil representatives of this group (e.g., Dipterus), and the usual occurrence of one to several large median elements in this region, make it difficult or impossible to homologize the dermal bones of the skull with those of amphibia. The characteristic dentition is far removed from that of the amphibia. Marginal teeth, with exceptions in the cases of some very early forms (e.g., Phaneropleuron), are lacking; in the later forms the loss of maxillae, premaxillae and nasals shows a progressive tendency toward degeneration in these regions. The concen-

tration of the teeth into tritoral clusters in the roof and floor of the mouth is not in itself an unfavorable feature, for vomerine teeth occur in the amphibia; but in all except certain very early fossil forms (e.g., Uronemus) the fusion of these teeth into large dental plates with grinding ridges has gone too far to give rise to the condition found in the amphibia. So far as the paleontological and anatomical evidence is concerned, the known facts tend to exclude the dipnoi from the direct ancestry amphibia, of the yet do not wholly preclude the possibility that future discoveries may supply us with more favorable material amongst early fossil forms. In so far as the terrestrial adaptations of the dipnoi resemble those of the amphibia, the case may be one of parallelism or convergence; their more fundamental resemblances indicate that they are not very far removed from a common ancestry.

Turning to the crossopterygii we find more favorable anatomical and paleontological grounds for comparison with the amphibia. The dermal elements forming the roof of the skull occur in paired series; a large number of cranial bones may be definitely homologized with those of the amphibia (see especially Baur '96; Moodie '08 a; for materials for further comparison see Goodrich '09, and Zittel '11). It is difficult to believe that identical relations in so many bones could have been independently evolved. With regard to the fins, we find examples of a bifurcated type of endoskeleton that makes a more favorable starting-point for a tetrapod limb than the archipterygial type of the dipnoi; e.g., see fig. 203 for the pectoral fin of Sauripterus, and Goodrich '09, p. 275, fig. 244, for the pelvic fin of Eusthenopteron; the pectoral fin of Eusthenopteron (Goodrich '09, p. 282, fig. 252) is not quite so favorable. But both of these forms belong to the Rhizodontidae, whose skull is not so favorable for comparison with the amphibia as the skull of some other crossopterygians; in no one form do we find all the conditions ideal for the derivation of the tetrapod type. The occurrence in Polypterus of two kinds of ribs, both the ventral or pleural ribs characteristic of the teleostomi and dipnoi, and the dorsal ribs characteristic of elasmobranchs and tetrapods, is a point emphasized by Baur

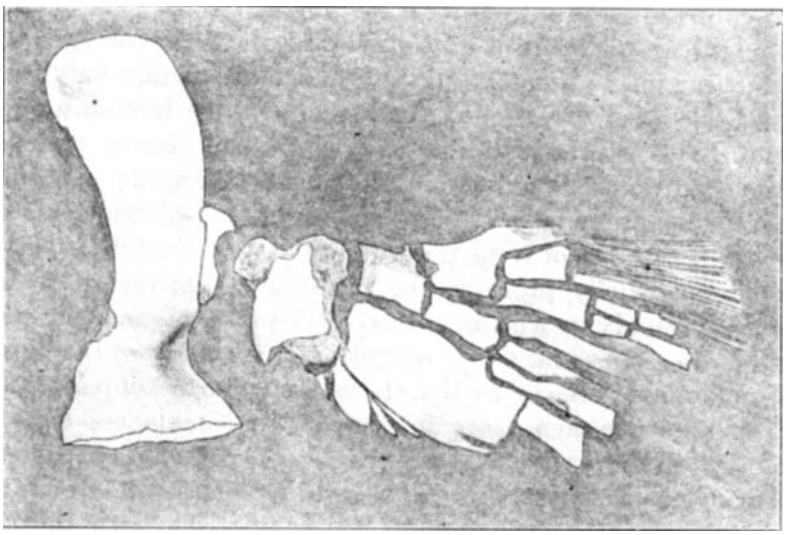


Fig. 203 Endoskeleton of the pectoral fin of Sauripterus taylori Hall, a crossopterygian from the upper Devonian. One-third natural size, linear reduction. From a drawing by Dr. L. Hussakoff of the American Museum of Natural History.

('96) as favoring a crossopterygian rather than a dipnoan ancestry for the amphibia.

It is upon embryological grounds that the strongest case has been made out for the derivation of the amphibia from the dipnoi; the known facts of development indicate the common origin and later separation of these two groups. In so far as this view is based upon a study of the early stages, the evidence may be dismissed with the remark that the early development of the crossopterygian Polypterus (Kerr '07 a) resembles that of the anura and the urodeles quite as much as does the early development of the dipnoi (Semon '00 and '01; Budgett '01; Kerr '00, '01 and '09); furthermore that these early stages are of very little value in connecting up the great groups of vertebrates. But some marked resemblances between dipnoi and amphibia in the later stages of development cannot be disregarded. Kellicott ('05, a and b), on the basis of a detailed study of the circulatory system of Ceratodus, came to the conclusion: "The resemblance in the vascular and respiratory systems between Ceratodus, the

most primitive of the dipnoi, and the amphibia, especially the urodeles, are numerous and important, and cannot be explained as parallelisms." In this connection it is important to compare the development of the circulatory system of Polypterus. From the account given by Kerr ('07), it appears that in Polypterus the vascular system, particularly because of the presence of only a single pair of aortic arches, is decidedly less amphibian in character than that of Ceratodus.

In the present state of our knowledge it is impossible to reach an unqualified decision of the question under consideration. In weighing the evidence one should not forget that in numerous cases where anatomical and embryological evidence have come into conflict in deciding questions of the phylogenetic relationships of the larger groups of animals, it is the embryological evidence that has had to give way, and that recent anatomical evidence has to give place to paleontological. Whatever light may be shed by future discoveries on the question of the derivation of the amphibia from the crossopterygii or the dipnoi, it is clear that the point of origin is not far from either stock; in other words, that the three lines of descent have separated from a common stem at no very great intervals.

Concerning the immediate ancestry of the living amphibia we have detailed evidence only in the case of one group, which fortunately for our purpose is the caudata or urodela. According to Moodie ('08 b) the urodeles are descended from the branchiosauria, a group of primitive extinct amphibia from the carboniferous and Permian. These are small, short-tailed amphibians with broad heads. The skull is slightly more complex than in the urodeles, and there is a dermal exoskeleton consisting of rows of thin semi-cycloid scales, especially on the flanks and under side of the body. External gills are present in the larvae. The view that these forms are ancestral to the urodeles is based on a detailed comparison of the structure of the skull, the structure and form of the vertebrae and the ribs, the number of digits, the arrangement of the phalangeal elements, the character of the pectoral and pelvic girdles, the distribution of the lateral line system, the structure and form of the long bones, and finally

the shape of the body. According to Moodie the caudata are degenerate branchiosaurians and the changes which have taken place in the exoskeleton are mostly brought about by the loss of certain parts. The urodele skull is especially degenerate in the occipital and temporal regions; it may be derived from the skull of Branchiosaurus (for which see Credner '81 to '90) by the loss of the dermoccipitals (supraoccipitals of Moodie '08; postparietals of Zittel '11), supratemporals (Zittel '11), postfrontals, postorbitals, sclerotics, epiotics (tabulares of Zittel '11), jugals and quadratojugals. The urodele skull has also in many cases become narrow in connection with a general elongation of the body—a degenerate feature. Hand in hand with the loss of dermal elements in the skull has gone the loss of the exoskeleton of the body.

The paleontological history of the apoda is unknown; but existing members of the group are in certain respects more primitive than the urodeles and more nearly allied to the stegocephali. Thus the hyoid and branchial apparatus is more primitive than that of any other recent amphibia; dermal scales are present which are probably homologous with those of the stegocephali. As in the urodeles, the skull shows degeneration in the loss of certain bones; but the epiotics (tabulares) are retained, and occasionally the postfrontals and the lacrimals. A second row of teeth is sometimes present on the mandibles. Aside from some very degenerate features, in other respects the apoda are highly specialized, indicating, as in the case of the anura, a line of descent separate from the urodeles. Yet there are some very suggestive resemblances. Attention has already been directed (Smith '12) to the marked similarity in structure of the egg envelopes of Ichthyophis to those of Cryptobranchus and Amphiuma, and to the likeness in the brooding habits; but in Ichthyophis the female protects the eggs, in Cryptobranchus the male. In their embryological development the apoda show many points of similarity to the reptiles.

With this background, we now come to the question of the interrelationships of the urodeles. In particular we are concerned with the phylogenetic position of the aquatic urodeles (the peren-

nibranchs and the derotremes[2]) as related to the land-living salamanders.

The most prevalent view has been that the aquatic urodeles are the most primitive. Parker and Haswell ('97, vol. 2, p. 291) have said: "The perennibranchiate urodeles are undoubtedly the lowest of existing amphibia; they lead up, through such forms as Amphiuma, with persistent gill slits but deciduous gills, to the land salamanders, in which a purely terrestrial form is assumed." In a group standing between fishes and the typically terrestrial vertebrates, it is natural to regard the aquatic forms as transitional to the terrestrial. In fishes there are usually five branchial arches, and the gill slits remain open throughout life. The land-living salamanders have in the adult state only two branchial arches (Parker '77; Wiedersheim '77; Cope '89), and the gill slits are open only to the end of larval life. In the aquatic urodeles there are usually three or four branchial arches (as in the larvae of the terrestrial forms), and the gill slits usually remain open throughout life—conditions intermediate between fishes and salamanders. In view of the occurrence of external gills in all larval forms, the persistence of such gills in the perennibranchs might, on the recapitulation theory, be regarded as a primitive character.

In the opinion of various authors, the above interpretation represents a short-sighted view of the matter. Boas ('81) was the first to assert that the perennibranchs are larvae that have lost the ability to transform; this conclusion was reached as a result of a comparative study of the circulation. Cope ('85) described the retrograde metamorphosis of Siren, and concluded that the present Sirens are descendants of a terrestrial type. Gadow ('01, p. 66) suggested a terrestrial ancestry for the urodeles. Kingsley ('01) says: "The salamandrina form the central urodele stem, and the perennibranchs and derotremes have been derived from this stem by degeneration and the retention of larval characters." Kingsbury ('05), on the basis of a compar-

[2] The classification of Stannius ('56) is here followed, as it seems best adapted for the purposes of this discussion.

ative study of the cranial elements, came to the tentative conclusion that Necturus is a permanent larva. The generalization formulated by Boas has recently been reiterated and expanded by Versluys ('09); briefly stated, his views are as follows:

The great resemblance of the perennibranchs to salamander larvae is only a consequence of the fact that the first are also larvae, but larvae which no longer come to full development as their ancestors did; the metamorphosis is imperfectly undergone or wholly omitted. Nevertheless these larvae become sexually mature, as in neoteny. In the course of time, in adaptation to their aquatic habitat, they have become degenerate in many respects. The derotremes are salamanders that have become fixed in the transitional stage or metamorphosis. Thus the aquatic urodeles have a terrestrial ancestry; they are forms which have reverted to an aquatic mode of life.

The probable course of events giving rise to the perennibranchs may be described as follows: While the mature salamanders are constructed after the fashion of a land animal, their larvae live in water and in the course of time have become more and more adapted to aquatic life. They have extended their larval organization and thus increased the difference, which must be overcome by metamorphosis, between the larva and the grown-up animal. In time some organs show arrested development or degeneration of such a sort that the larvae can no longer develop into land-living salamanders; they remain life-long water dwellers. The condition is one of fixed neoteny (Boas '96). According to this view the perennibranchs are disconnected from one another and have evolved as neotenic larvae from different salamanders.

Confirmatory evidence for this view comes from the studies of Emerson ('05) on Typhlomolge. In some details of its structure this animal shows a remarkable similarity to the larvae of Spelerpes ruber. Probably Typhlomolge has been derived from the neotenic larva of some salamander of the family (Plethodontidae) to which Spelerpes belongs, adaptations to a subterranean water life having been added.

The derotremes also are not primitive urodeles. Since they show a mixture of the characters of the larvae and grown-up salamanders, one cannot derive them from typical larvae. Presumably the derotremes are descended from typical salamanders which have returned completely to aquatic life. Thus the metamorphosis, which serves to adapt the larvae to land life, has lost its biological meaning; it extends over a longer time, and finally sexual maturity overtakes the yet imperfectly built animal. Some organs complete the metamorphosis, others retain wholly or in part the larval condition. So in its skull Cryptobranchus (Reese '06) resembles the grown-up salamander; in its circulation it retains larval characters. Versluys believes Cryptobranchus to be descended from the amblystomidae.

If we take into account only living forms, much of the data thus far considered in seeking a solution of this general question of the relationship of the aquatic and land-living urodeles may be read either way. The key to the situation lies in the comparison of the structures of existing urodeles with those of fossil forms. We have seen that the extinct ancestor of the urodeles was probably an animal whose skull was more complete than that of any living urodele. In Necturus (Kingsbury '05), there is a considerable reduction in the number of cranial bones, comparable to the condition in the larval Spelerpes, and contrasting with the condition in the adult Spelerpes. If the stem-form of the urodeles was an animal like Branchiosaurus, then so far as the cranial elements are concerned the salamander, and not the perennibranch, is the more primitive form. There are five branchial arches in Branchiosaurus—a condition which does not exclude either view of the interrelationships of the urodeles. Branchiosaurus had external gills only in the larval stage; this indicates that the condition found in the perennibranchs is probably not in the ancestral line of the caducibranchs (salamandrina).

Von Eggeling ('11), from a comparative study of the histogenesis of the skeleton of the limbs of urodeles, favors the view that the perennibranchs, derotremes and siredon are derived from the caducibranchs.

With the transition from the water to the land, or vice versa, one might expect important modifications in the sense organs. In the amphibia this expectation is best realized in the auditory organs, which in the form of a columellar apparatus fitting into a fenestra ovalis here occur for the first time in the vertebrate series. From a study of the modifications of this apparatus we may well hope to obtain information concerning the more intimate phylogenetic relationships within the group. According to the recent work of Kingsbury and Reed ('09) on the urodeles, there is close correlation between the type of auditory apparatus present and the habits (aquatic, semi-aquatic, terrestrial, burrowing, etc.) of the animals. From their results one may note many resemblances between the adults of the typically aquatic forms and the aquatic larvae of the terrestrial forms, as well as contrasts between both of these and the adult terrestrial forms. Concerning the phylogenetic relationships as indicated by the auditory apparatus Reed ('09) says:

> Cryptobranchus is the most generalized. The amblystomidae are intermediate between Cryptobranchus and all other groups. The plethodontidae and desmognathidae are departures from the Amblystoma stem while from these the sirenidae and Amphiuma seem to be degenerated. Diemyctylus and Triton are identical with regard to these ear structures and differ from all others. They are to be considered the most specialized. Between Diemyctylus and Triton on the one hand and the amblystomidae on the other Salamandra stands intermediate, resembling more strongly the amblystomidae.

If the generalized condition is really a primary one, then so far as the evidence from this single character is concerned, Cryptobranchus is one of the most primitive of urodeles; the evidence is not in line with the hypothesis of Versluys. But one should seriously consider whether the correlation between the sound-transmitting organs and the environmental relations is not too close to make the character of much phylogenetic value.

Osborn ('88) states that in Cryptobranchus we have the most primitive type of brain thus far observed among the amphibia. But it is not clear that the simple condition found is necessarily primitive.

Whipple ('06), from a comparative study of the ypsiloid apparatus in urodeles reaches the following conclusions:

(a) "That forms with lungs but without vestiges of an ypsiloid apparatus, and with no evidence of degeneration in the pelvic region (e.g., Necturus) are neither degenerate forms, nor permanent larvae of any of the salamandrina."

(b) "That the presence of a functional ypsiloid apparatus in Cryptobranchus indicates that Cryptobranchus lies near the line of descent of the salamandrina."

In summing up the facts for and against the hypothesis of the phylogenetic relationships of the perennibranchs, derotremes and salamandrina as outlined by Versluys, it seems to me that the arguments in favor of the hypothesis are founded on characters of greater phylogenetic value. In the reptiles and mammals, land forms are always primitive, aquatic forms secondary (Osborn '02). To the writer the evidence seems convincing in favor of a similar view for the recent urodeles. It might be added that pentadactylous limbs, more or less perfectly developed in fossil as well as recent amphibia, were undoubtedly produced in connection with terrestrial habits; and it should be emphasized that the forms ancestral to the present-day aquatic urodeles were probably not purely terrestrial, but passed through an aquatic larval stage, as in Branchiosaurus and most of the living salamanders.

It remains to consider briefly the phylogenetic value of some of the facts concerning the life cycle of Cryptobranchus that are embodied in the present contribution, and to discuss their bearing on the subjects just treated from a historical point of view. In an investigation thus far confined mainly to the external features of development, manifestly little more than a beginning can be made in such an interpretation.

The repeated failure of embryological generalizations to solve some of the larger phylogenetic problems led to a widespread reaction against the earlier too sweeping conclusions based on the recapitulation theory. The reproductive processes, while so fundamental, are very plastic, modified in closely related species and even changing somewhat in the same species kept under

different conditions. A strict application of the biogenetic law would imply that the early stages of development are exclusively palingenetic; but from the earliest stages we find adaptations (e.g., the presence of yolk) that are prospective in their significance and have to do with distinctively larval phases, while the larval characters may be highly coenogenetic. It is the failure to distinguish between palingenetic and coenogenetic features of development that is mainly responsible for bringing the recapitulation theory into disrepute. With a clearer recognition of the limitations in this field of study, we may yet question whether the reaction against the validity of the recapitulation theory has not gone too far. As compared with anatomy and paleontology, embryology is doubtless of little service in connecting up the great groups of animals; yet it is indispensable in the solution of many special problems.

In a comparative study of the breeding habits (Part I, Smith '12) we must remember that we are dealing with characters that occur very late in the ontogeny, having to do with the adult rather than with the developing animal. Consequently such characters are of no more phylogenetic value than the habits in general and the family, generic and specific morphological characteristics of the adult; they are exceedingly plastic and of value for comparison only within a very limited range of forms.

A phylogenetic interpretation of the methods of fertilization in urodeles has been given in a previous paper (Smith '07 b); it is here referred to with the remark that in view of the conclusions reached in the present paper regarding the trend of evolution in the urodeles, the series should be reversed. If we could go back far enough in the phylogeny of the vertebrates, doubtless we should find external fertilization to be the primitive condition (e.g., as in Amphioxus); but it is entirely possible that in Cryptobranchus the method is secondarily acquired.

As we should expect, the brooding habit of Cryptobranchus is very similar to that of the closely-related Amphiuma. The absence of a brooding habit in Necturus is noteworthy. Brooding habits very similar to that of Cryptobranchus are found in Desmognathus and Plethodon, but with this important differ-

ence: in these latter forms the female, not the male, cares for the eggs.

The egg capsules of the urodeles, as in other groups, show generic and even specific differences (e.g., the specific differences in the egg masses of Amblystoma, Smith '11 b). This is what might be expected, since the capsules are the product of the soma, not of the germ cells; the facts are in no way incompatible with von Baer's law. The close resemblance between the egg envelopes of Cryptobranchus and Amphiuma accords with the systematic relationship. Amongst terrestrial urodeles there is no close approach to the type found in Cryptobranchus; but in general we should seek for affinities in forms having the egg capsules more or less independent in the cluster, connected by stalks (e.g., as in Desmognathus, Wilder '99), rather than in forms in which the individual capsules are surrounded by a common jelly mass, as in Amblystoma.

The origin of the follicle cells of Cryptobranchus has been traced (Part I) from the epithelial cells of the ovarian wall. Hence these cells belong to the soma, and the rather marked differences between the follicle cells of Cryptobranchus and Necturus are of value for comparison only with a very limited range of forms.

Taking up the history of the egg proper, we first note that the progressive change of the ovarian egg from an alecithal through an isolecithal to a telolecithal stage is a recapitulation of a very ancient series of events in the phylogeny. The actual amount of yolk present is a coenogenetic character, for the yolk content changes greatly within nearly related forms.

The factors that determine the amount of yolk present in the eggs of different species are complex and do not readily fall under any single law. Protection of the eggs through nesting and brooding habits makes possible a reduction in their number, enabling the female to endow each egg with a larger store of yolk, thereby giving the young a better start in life. Such a store of yolk allows development to go further before the young animal is cast upon its own resources, so that the necessity for peculiarly larval adaptations is minimized. In purely terrestrial

non-placental forms a large amount of yolk is certainly necessary for the food (e.g., insect larvae) of the young is larger and less easy to capture than is the case with aquatic larvae. On the other hand a large store of yolk is common in the eggs of fishes. The most we can infer is that the presence of a large amount of yolk in the egg is one of the conditions that makes possible the invasion of the land; it cannot be said that the presence of an unusual amount of yolk in the egg of Cryptobranchus is evidence either for or against a terrestrial ancestry.

Among the known eggs of urodeles that of Cryptobranchus is probably the most heavily yolk-laden; the egg of Necturus contains nearly as much yolk. In the salamandrina one notes the large amount of yolk in the eggs of Desmognathus (Wilder '04; Hilton '04 and '09); Spelerpes (Goodale '11) and Plethodon (Piersol '09).

The absence of pigment in the egg of Cryptobranchus is correlated with the nesting habits, whereby the eggs are protected from the light. Similar conditions are found in Necturus, Desmognathus, Plethodon and Spelerpes.

The occurrence of a protoplasmic mantle and cytodisc in the late ovarian egg of Cryptobranchus parallels a condition in the teleost egg which reaches its full expression just before fertilization; in Cryptobranchus this condition is transient. The segregation of a definite layer of cytoplasm close to the surface of the blastodisc in Cryptobranchus shortly after fertilization suggests a parallel with the marked increase in thickness of the germinal disc of the teleost egg immediately after fertilization; Professor Dean informs me that he has observed a similar phenomenon in ganoids.

The fundamental features of the early embryonic development have to do with the building up of the very general structures and body relations common to all vertebrates. In their most general aspects cleavage and gastrulation are extremely palingenetic phases of development. But we find secondarily imposed on the essential features of these processes, modifications which are highly coenogenetic and of adaptive significance mainly for the embryo and larva (Lillie '98), rather than for the adult.

These stages seem to possess few characters intermediate between the extremes indicated, consequently they tell us little about the relationships of the larger groups; but the coenogenetic characters, which are plastic and vary widely within a limited range, may be of value for determining relationships within these groups. The most striking of these coenogenetic characters are correlated with the presence of yolk (Conklin '07).

In the highly telolecithal and heavily yolk-laden egg of Cryptobranchus, we may interpret holoblastic cleavage as a persistent primitive character. For were the ancestral form one with meroblastic cleavage, we should hardly expect the holoblastic method to arise under such unfavorable conditions.

Comparisons between the early cleavage furrows of different urodeles seem justified on the ground that we are comparing cells of the same generation; but in view of the highly indeterminate character of the cleavage (Jordan and Eycleshymer '94) such comparisons do not take us very far. Mechanical factors doubtless play a part (McMurrich '94), but these mechanical factors are conditioned by the organization of the egg, which is hereditary.

There is close correlation between the method of third cleavage and the yolk content. A vertical third cleavage is characteristic of heavily yolk-laden and highly telolecithal eggs; a latitudinal third cleavage is found rather in eggs with yolk both smaller in amount and with a lesser degree of segregation from the cytoplasm. Morgan ('93) has shown that in teleost eggs from which yolk has been experimentally removed, the third cleavage often comes in latitudinally, yet the eggs produce perfect embryos. Marked variation in the direction of the third cleavage furrows occurs in eggs in which the conditions are intermediate in character; the egg is oscillating between two possible modes of cleavage. A vertical third cleavage is characteristic of the egg of Cryptobranchus; it is found less uniformly in the eggs of Desmognathus; in Necturus and Diemyctylus the third cleavage is irregular; in Amblystoma it is latitudinal. So far as this character is concerned Cryptobranchus lies nearest to Desmognathus and is most remote from Amblystoma.

The important features of the later stages of the cleavage have to do with processes that are not well expressed in the superficial cleavage pattern: migration of cells and the various processes of differentiation that lead up to gastrulation and early embryo-formation.

The occurrence of a septal furrow in the gastrula stages of two such widely-separated forms as Cryptobranchus and Petromyzon is a remarkable case of convergence in purely embryonic characters. The septal furrow and fenestra are the mechanical product of gastrulation by invagination and epiboly in a heavily yolk-laden egg with a very thin roof to the blastocoele. So far as known these features of gastrulation in Cryptobranchus are unique among urodeles; but there is evidently an approach to this condition in Spelerpes, since the egg is heavily yolk-laden and during gastrulation the blastocoele roof becomes quite thin (Goodale '11).

The study of the later embryonic and larval stages is as yet too superficial to furnish much data for phylogenetic generalization; yet for this purpose the late stages will probably prove of greater value than the earlier ones (Wilson '98, p. 23). It should be particularly noted that the larval Cryptobranchus reaches an age of two years before transforming—evidence of a retarded metamorphososis. Reasons have already been given for believing that the metamorphosis is incomplete.

The study of the breeding habits, the organization of the eggs, and the early course of development lead us to look among the land-living salamanders for affinities to Cryptobranchus—more particularly to forms like Desmognathus, Spelerpes and Plethodon. Considerable evidence from comparative anatomy, particularly with regard to skull structure, will be found to harmonize with this view. Desmognathus in particular is suggestive; according to Kingsbury ('02) it is semi-aquatic in its habits, living at the edges of swiftly running brooks. It conceals itself under stones at the edge of the stream or in its immediate vicinity, and here its unpigmented and heavily yolk-laden eggs are laid. There is a brooding habit, though in this case the female guards the eggs; we have noted some marked similarities to Cryptobranchus in the early development. Yet we are hardly warranted in con-

cluding that the relationship is very close. In particular we should expect the immediate ancestral stock of Cryptobranchus and the closely related miocene fossil Andrias scheuchzeri (Gadow '01, p. 84) to consist of larger animals.

According to the view adopted in this paper, the urodeles, very remotely descended from aquatic stock, are primarily terrestrial, but with aquatic larvae. On the land they were unsuccessful in the struggle for existence in the open; they took refuge in sheltered situations, and for this they have paid the penalty of degeneration. Yet, in the main, the result of the arrest of a typical terrestrial adaptive radiation has been the retention of primitive characters. Some became secondarily aquatic; this is one phase of the tendency toward secluded habits, and involves the retention of larval characters. Added to these more conspicuous peculiarities we find a great variety of special adaptations to a retired mode of life, some of them correlated with the defenseless condition of the animals. Of all these changes, reversion to an aquatic mode of life is a factor which, cutting across many lines of descent, has done most to disguise the real relationships, and of this we have a conspicuous example in Cryptobranchus.

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# PLATES

## PLATE 3

### EXPLANATION OF FIGURES

(Cryptobranchus allegheniensis)

All the figures are from preserved material, fixed in bichromate-acetic-formalin excepting the eggs shown in figures 210 and 212 which were fixed in formalin. The animal hemisphere is shown in every case. × 4.

204 Stage 1. First cleavage.

205 Stage 2. Second cleavage.

206 Stage 3. Third cleavage.

207 Stage 4. Fourth cleavage.

208 Stage 4. Fourth cleavage. Figure 82 is drawn from the same egg.

209 Stage 4. Fourth cleavage. Figure 81 is drawn from the same egg.

210 Stage 5. Thirteen micromeres. Figure 87 is drawn from the same egg.

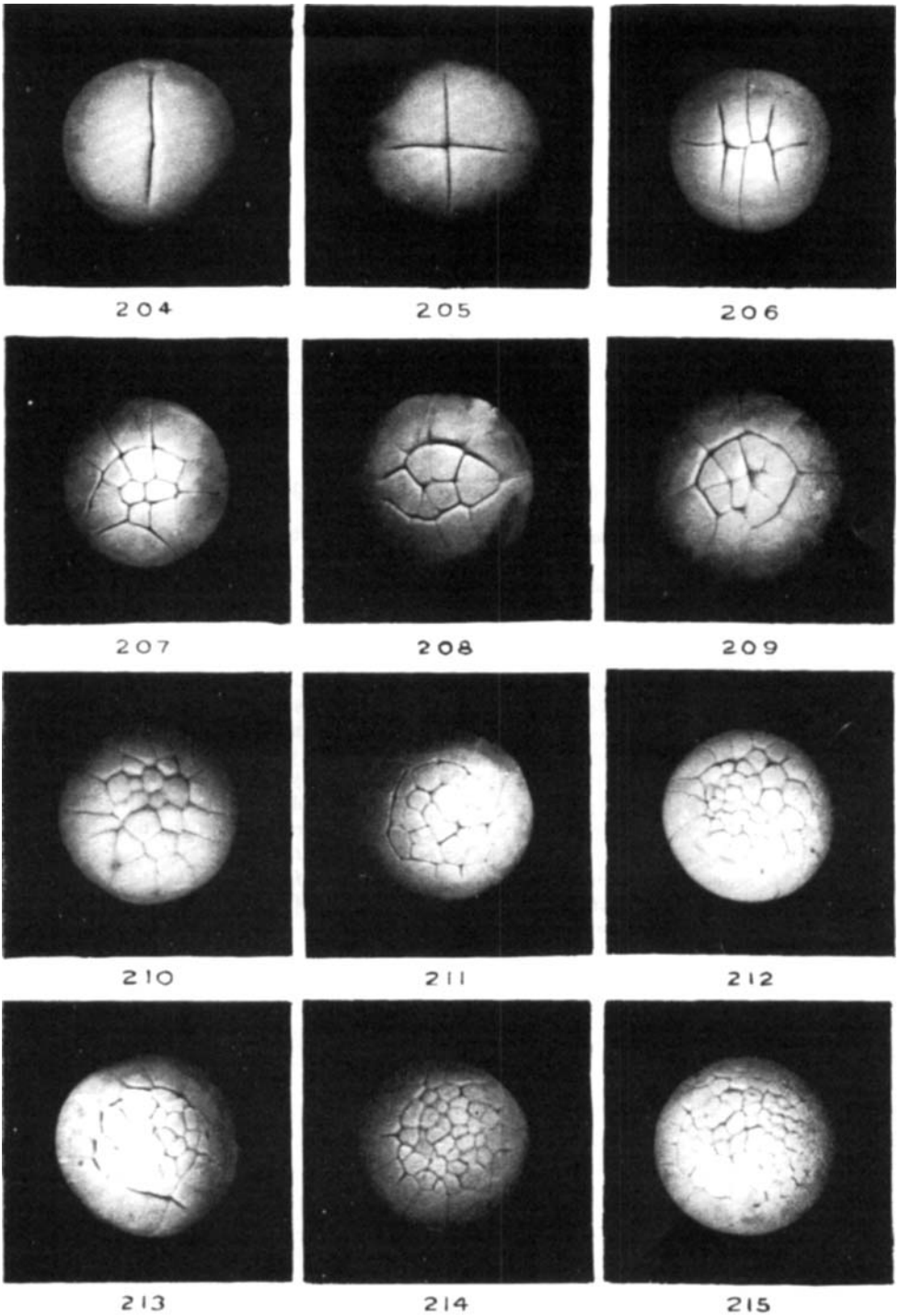
211 Stage 5. Seventeen micromeres. Figure 86 is drawn from the same egg.

212 Stage 6. About thirty-two micromeres. Figure 98 is drawn from the same egg.

213 Stage 6. About thirty-two micromeres.

214 Stage 6. About thirty-six micromeres.

215 Stage 7. About sixty-four micromeres.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## PLATE 4

### EXPLANATION OF FIGURES

(Cryptobranchus allegheniensis)

All the figures are from preserved material fixed in bichromate-acetic-formalin. × 4.

216 Stage 7.5. Equatorial view.

217 Stage 8.0. Upper hemisphere.

218 Stage 9.5. Equatorial view.

219 Stage 10.5. Equatorial view of an embryo nearly ready for gastrulation.

220 Stage 11.4. Early gastrula, showing the dorsal lip of the blastopore.

221 Stage 11.5. Antero-dorsal view of a gastrula, showing the fenestra.

222 Stage 11.6. Anterior view of a gastrula, showing the fenestra.

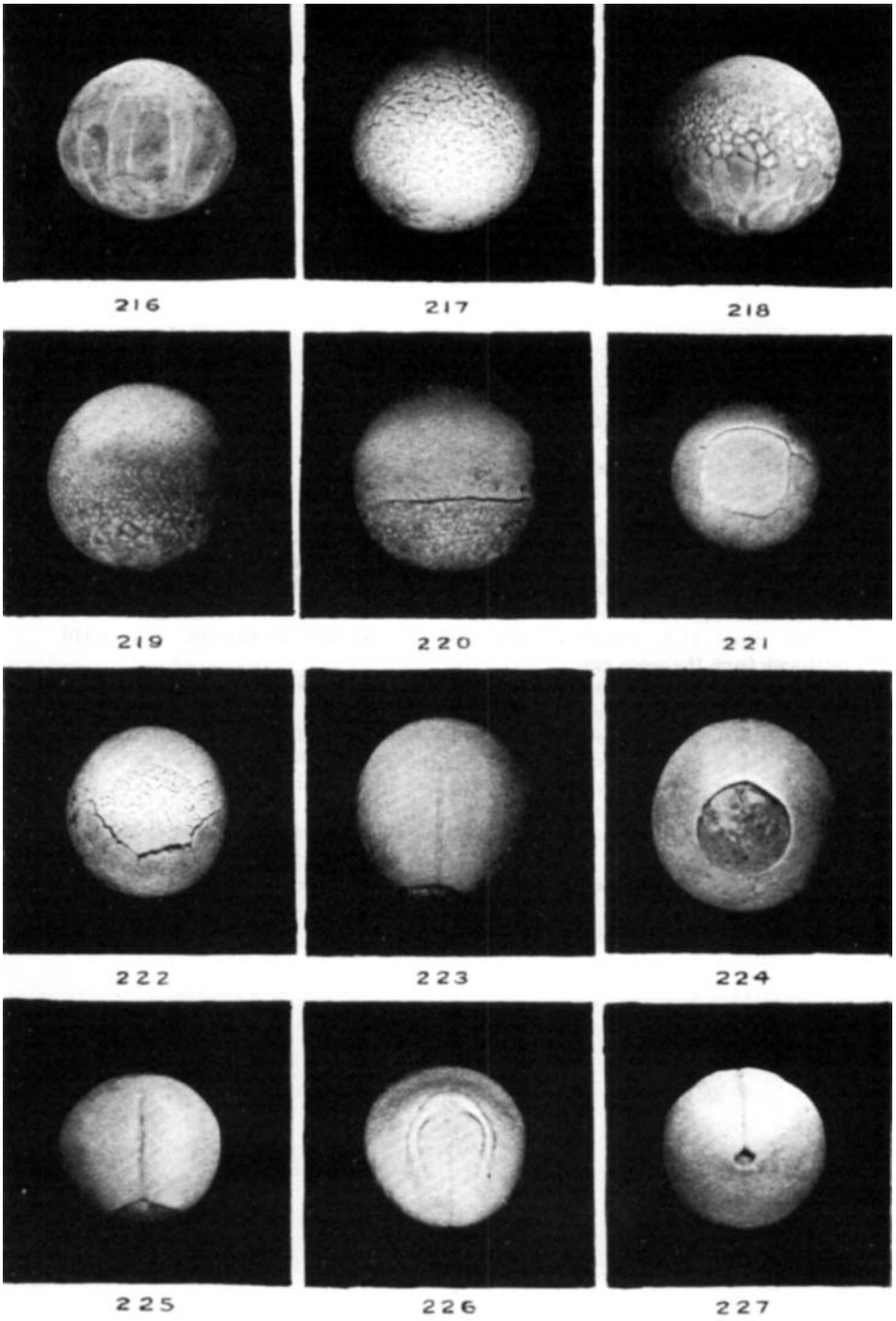
223 Stage 12.4. Dorsal view showing early neural plate and neural groove.

224 Stage 12.4. Posterior view of the same embryo as in the preceding figure, showing the yolk plug and a part of the neural plate and neural groove.

225 Stage 12.5. Dorsal view showing the neural groove and the dorsal lip of the blastopore. Figure 143 is drawn from the same embryo.

226 Stage 13.4. Dorsal view showing early neural folds.

227 Stage 13.5. Posterior view showing the form of the late blastopore, and the posterior part of the neural plate.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## PLATE 5

### EXPLANATION OF FIGURES

(Cryptobranchus allegheniensis)

Bichromate-acetic-formalin fixation. × 4.

228 Stage 13.5. Posterior view showing the form of the late blastopore, and the posterior part of the neural plate.

229 Stage 14.1. Dorsal view showing neural folds and the early segmentation of the neural plate. Figure 166 is drawn from the same embryo.

230 Stage 14.1. Postero-dorsal view of the same embryo as in the preceding figure, showing the late blastopore and the segmentation of the neural plate.

231 Stage 14.9. Posterior view, showing the late blastopore. Figure 176 is drawn from the same egg.

232 Stage 14.9. Dorsal view of the same embryo as in the preceding figure, showing the segmentation of the neural plate.

233 Stage 15.4. Dorsal view. Figure 184 is drawn from the same egg.

234 Stage 15.8. Dorsal view. Figure 182 is drawn from the same egg.

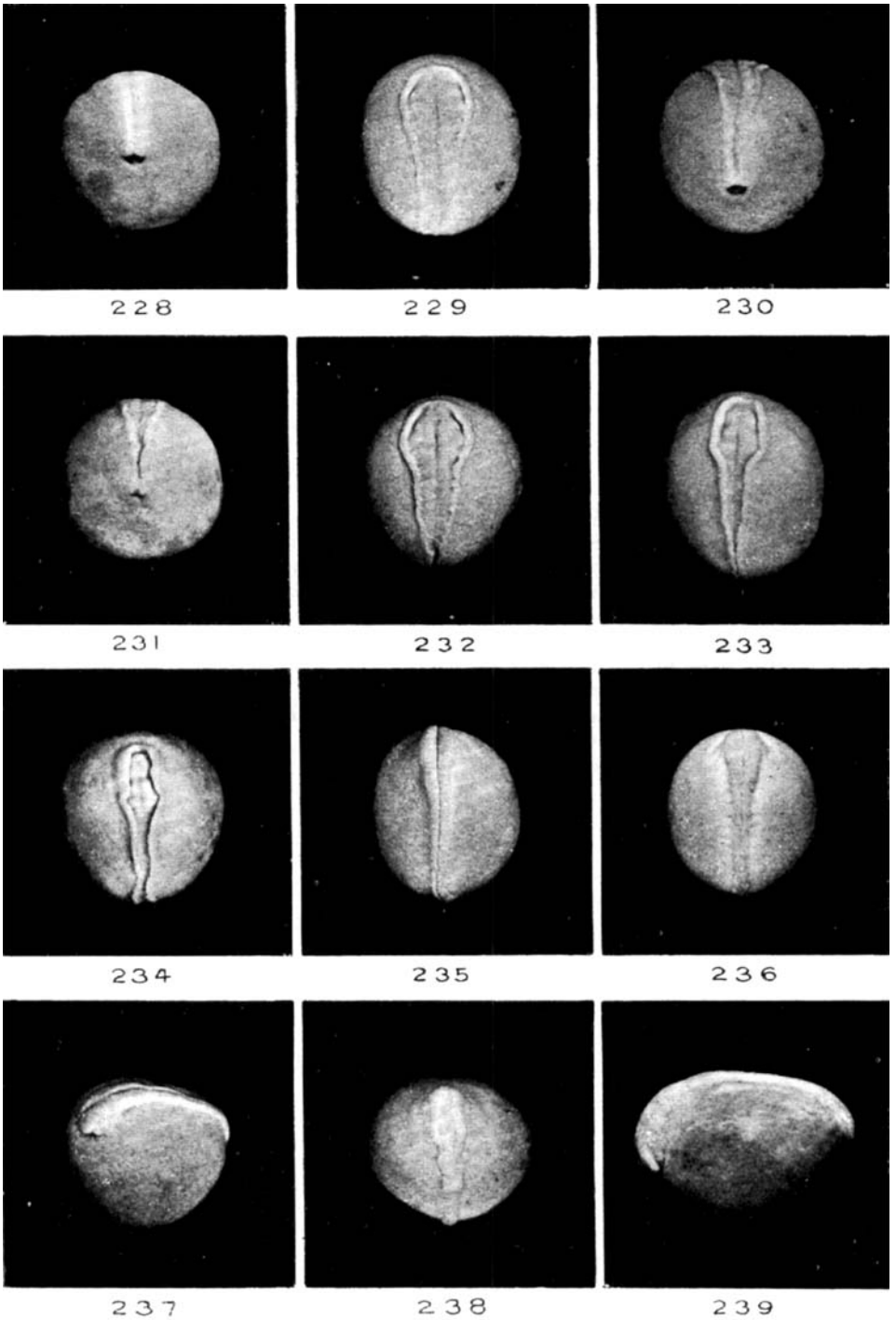
235 Stage 16.0. Dorsal view showing closing neural folds.

236 Stage 16.8. Dorsal view.

237 Stage 16.8. Antero-dorso-lateral view of the same embryo as in the preceding figure.

238 Stage 17. Antero-dorsal view (inverted with respect to the natural position).

239 Stage 17. Lateral view.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## PLATE 6

### EXPLANATION OF FIGURES

(Cryptobranchus allegheniensis)

Bichromate-acetic-formalin fixation. × 4.

240 Stage 17. Ventral view of the embryo shown in the preceding figure.
241 Stage 17. Dorso-lateral view of the embryo shown in the preceding figure
242 Stage 17. Dorsal view of the embryo shown in the preceding figure.
243 Stage 18. Ventral view.
244 Stage 18. Lateral view of the embryo shown in the preceding figure.
245 Stage 19. Lateral view.
246 Stage 19. Dorsal view.
247 Stage 19. Lateral view of the embryo shown in the preceding figure.
248 Stage 19. Lateral view.
249 Stage 20. Lateral view.

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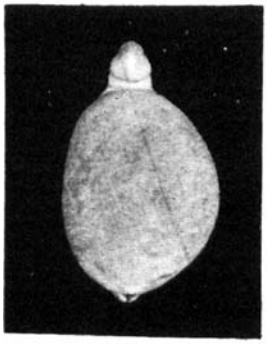

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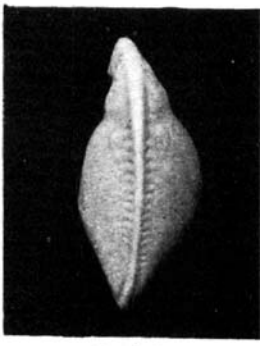

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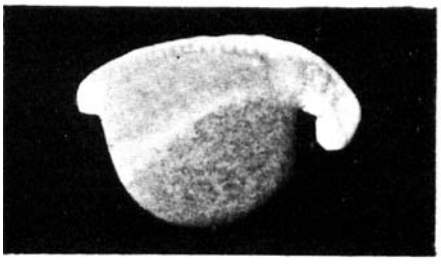

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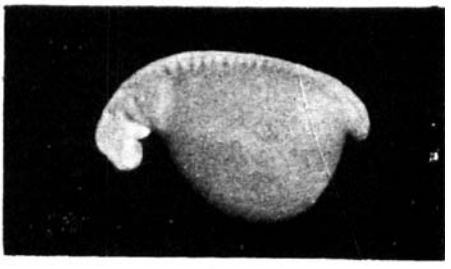

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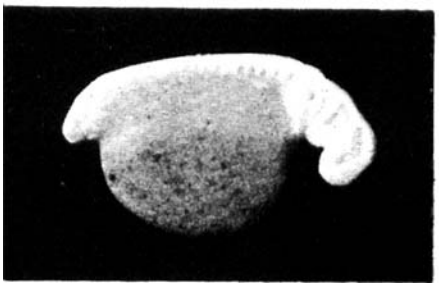
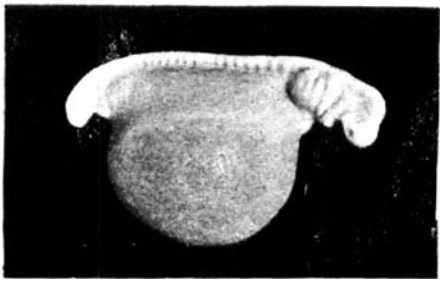




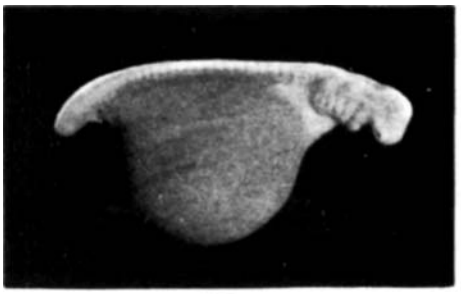
## PLATE 7

EXPLANATION OF FIGURES

(Cryptobranchus allegheniensis)

Bichromate-acetic-formalin fixation. × 4.

250 Stage 20. Lateral view.
251 Stage 20. Ventral view of the embryo shown in the preceding figure.
252 Stage 21. Ventral view.
253 Stage 21. Lateral view.
254 Stage 21. Dorsal view of the embryo shown in the preceding figure.
255 Stage 21. Ventral view of the embryo shown in the preceding figure.
256 Stage 22. Dorsal view.
257 Stage 22. Ventral view of the embryo shown in the preceding figure.



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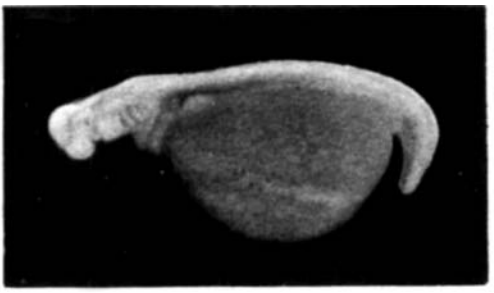

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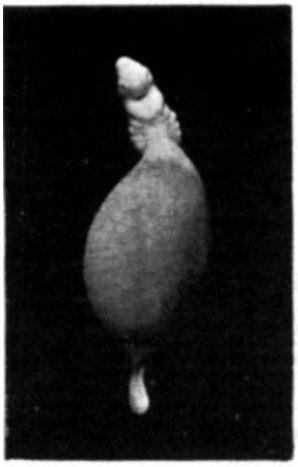

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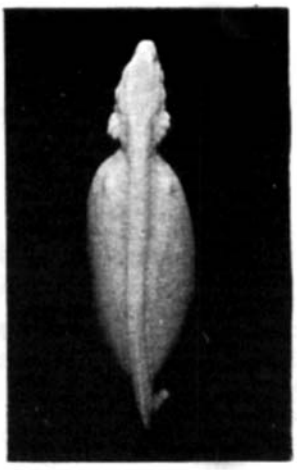

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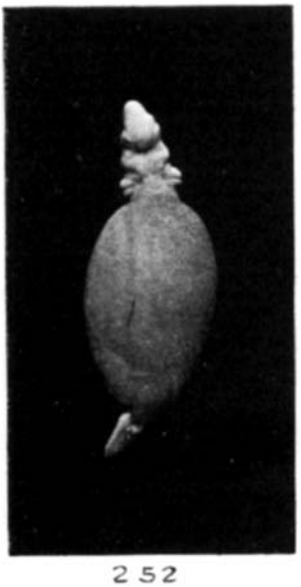

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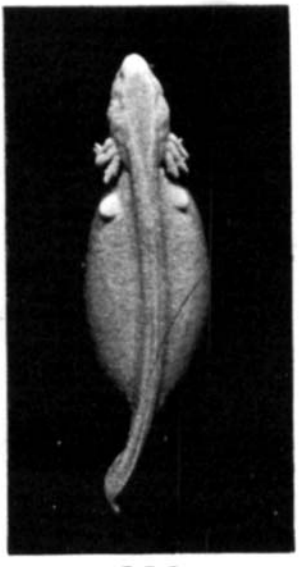

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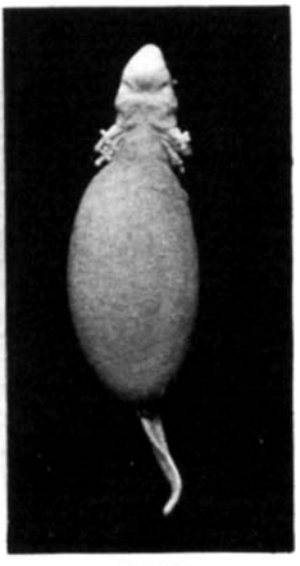


257

## PLATE 8

EXPLANATION OF PLATES

(Cryptobranchus allegheniensis)

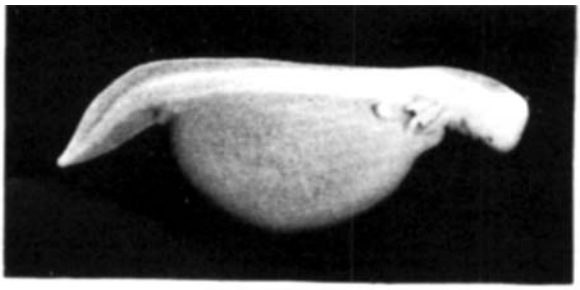
Bichromate-acetic-formalin fixation except for figures 260 and 261 which were made from living specimens anaesthetized with chloretone. × 4.

258 Stage 22. Lateral view.

259 Stage 22.5. Lateral view.

260 Stage 23. Dorsal view of an embryo ready to hatch.

261 Stage 23. Lateral (slightly ventral) view of a newly-hatched larva.



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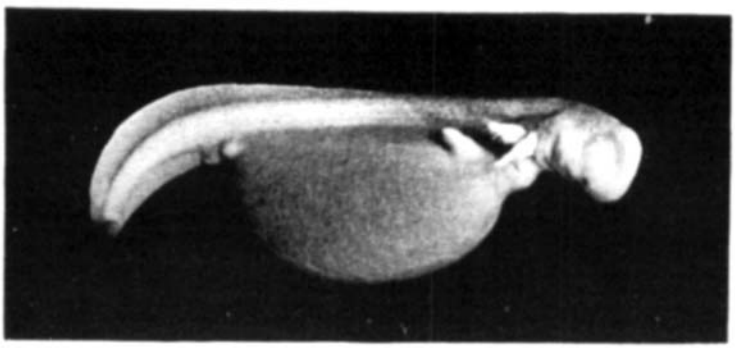

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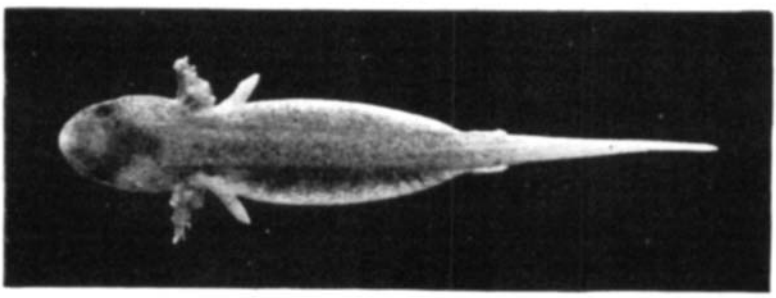

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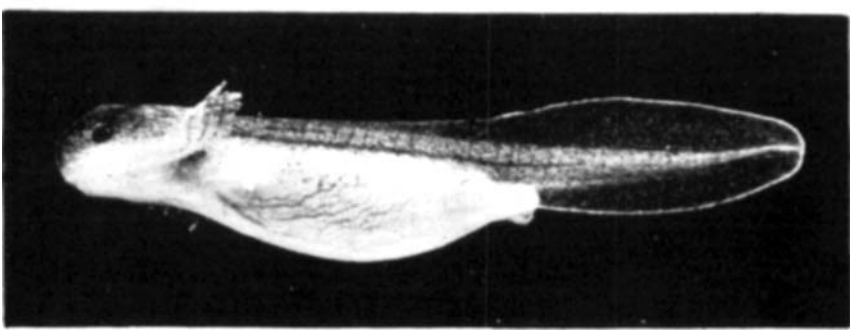


261

## PLATE 9

### EXPLANATION OF FIGURES

(Cryptobranchus allegheniensis)

All the figures are natural size.

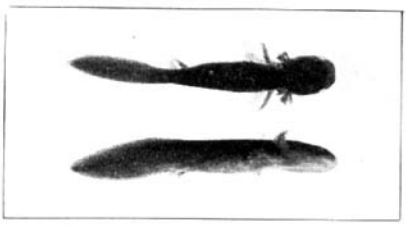
262 Living larvae reared in the laboratory, anaesthetized with chloretone and photographed about five weeks after hatching.

263. Larva reared in the laboratory, killed in bichromate-acetic-formalin about two months after hatching and preserved in formalin. The specimen is slightly shortened through the action of the fixing fluid.

264 and 265. Two views of a larva reared in the laboratory, anaesthetized with chloretone and photographed about ten weeks after hatching.

266. Larva reared in the laboratory, anaesthetized with chloretone and photographed about six months after hatching.

267. Year-old larva captured and fixed in formalin August 27, 1909; a few weeks later it was transferred to alcohol, in which it remained nearly a year before being photographed.



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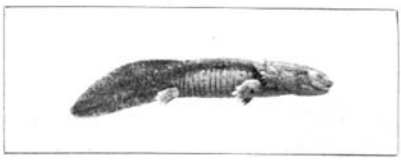
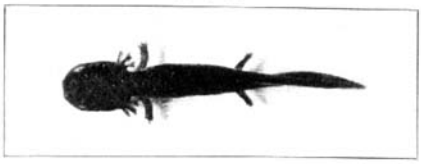


263



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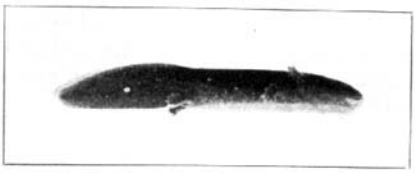

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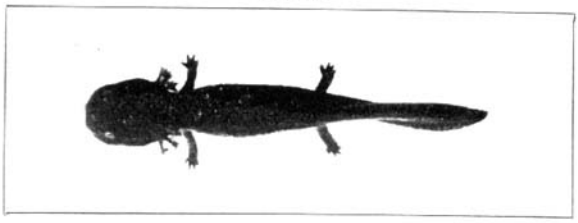

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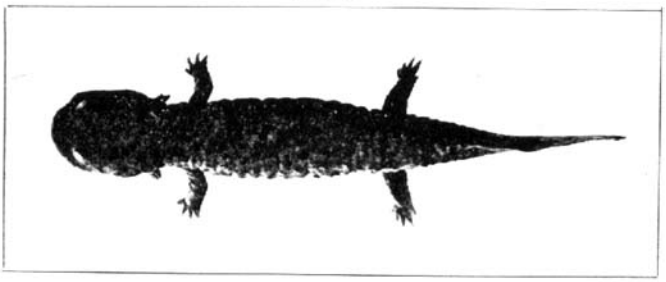

267

## PLATE 10

### EXPLANATION OF FIGURES

(Necturus maculosus)

The figures are intended to show in particular the late history of the blastopore. Figures 268 to 276 inclusive were drawn from the living eggs by Prof. Bashford Dean; figures 277 to 279 are from preserved material, and were drawn under the direction of the author by Miss Mabel L. Hedge.

*Necturus maculosus*